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# Analiza molekularna i funkcjonalna interleukiny 18 (IL-18), jej receptora (IL-18R) i białka wiążącego IL-18 (IL-18BP) w aktywnej gruźlicy i latentnym zakażeniu gruźliczym

Molecular and functional analysis of interleukin 18 (IL-18), its receptor (IL-18R) and IL-18 binding protein (IL-18BP) in active tuberculosis and latent tuberculosis infection

Praca doktorska

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# SPIS TREŚCI

# Wykaz publikacji będących podstawą rozprawy doktorskiej

1. **Wawrocki S.**, Druszczyńska M., Kowalewicz-Kulbat M., Rudnicka W. (2016). *Interleukin 18 (IL-18) as a target for immune intervention*. Acta Biochimica Polonica, 63(1), 59–63.
   https://doi.org/10.18388/abp.2015_1153

   **IF = 1,159; MNiSW = 15**

2. **Wawrocki S.**, Druszczyńska M. (2017). *Inflammasomes in Mycobacterium tuberculosis-driven immunity*. Canadian Journal of Infectious Diseases and Medical Microbiology, 2017, 2309478.
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   **IF = 1,165; MNiSW = 15**

3. **Wawrocki S.**, Kielnierowski G., Rudnicka W., Druszczyńska M. (2019a). *Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population*. Acta Biochimica Polonica, 66(3), 337–342.
   https://doi.org/10.18388/abp.2019_2797

   **IF = 1,626; MNiSW = 40**

4. **Wawrocki S.**, Seweryn M., Kielnierowski G., Rudnicka W., Włodarczyk M., Druszczyńska M. (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.
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   **IF = 2,776; MNiSW = 100**

5. **Wawrocki S.**, Kielnierowski G., Rudnicka W., Seweryn M., Druszczyńska M. (2020b). *Interleukin-18, functional IL-18 receptor and IL-18 binding protein expression in active and latent tuberculosis*. Pathogens, 9(6), 451.
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   **IF = 3,405; MNiSW = 100**

6. **Wawrocki S.**, Seweryn M., Kielnierowski G., Rudnicka W., Druszczyńska M. (2020a). *IL-18 and related function proteins associated with tuberculosis severity and screening for active TB among patients with non-mycobacterial community-acquired pneumonia (CAP)*. Saudi Journal of Biological Sciences, 27(11), 3035–3045.
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WYDZIAŁ BIOLOGII i OCHRONY ŚRODOWISKA Uniwersytet Łódzki

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Dotacje celowe na działalność związaną z prowadzeniem badań naukowych lub prac rozwojowych oraz zadań z nimi związanych, służących rozwojowi młodych naukowców oraz uczestników studiów doktoranckich w latach: 2017, 2018, 2019.

# Omówienie celu naukowego i uzyskanych wyników

## Wstęp

Gruźlica (TB), wywoływana przez prątki gruźlicy (*Mycobacterium tuberculosis* – *M.tb*), od lat pozostaje światowym problemem zdrowotnym, uznawanym za chorobę zakaźną o jednej z najwyższej śmiertelności, pomimo prowadzonych na szeroką skalę profilaktycznych szczepień przeciwgruźliczych. W polskim programie szczepień ochronnych, szczepienia przeciwgruźlicze żywymi atenuowanymi prątkami *Mycobacterium bovis* BCG (Bacillus Calmette-Guérin), są obowiązkowe od 1955 roku, a aktualnie obowiązujący kalendarz szczepień przewiduje podawanie BCG noworodkom jednorazowo już w pierwszych dobach życia (Fol et al. 2011; Nathavitharana and Friedland 2015; PZH 2020a, 2020b).

Według najnowszych danych Światowej Organizacji Zdrowia około 1,8 miliarda ludzi na świecie jest zakażonych prątkami gruźlicy (World Health Organization (Global Tuberculosis Programme) 2020). Zakażenie to może mieć bezobjawową postać utajoną (latentną), które jednak u co najmniej 10% zakażonych osób rozwija się w aktywną gruźlicę. Ryzyko rozwoju aktywnej choroby jest największe u osób z osłabioną odpornością, pacjentów z chorobami nowotworowymi i autoimmunizacyjnymi, którzy przyjmują leki o działaniu immunosupresyjnym, ale także u osób w starszym wieku (Furin, Cox, and Pai 2019; World Health Organisation 2018; World Health Organization (Global Tuberculosis Programme) 2020). W roku 2019 odnotowano na świecie 7,1 miliona nowych przypadków choroby, przypuszcza się jednak, że liczba ta jest znacznie niedoszacowana, a rzeczywista liczba zachorowań jest znacznie większa i może wynosić nawet 10 milionów przypadków (World Health Organization (Global Tuberculosis Programme) 2020). W roku 2019 z powodu gruźlicy zmarło około 1,4 miliona osób na świecie, w tym około 208 tysięcy z jednoczesnym zakażeniem *M.tb* i wirusem HIV. Narastająca liczba przypadków koinfekcji *M.tb* i HIV (*ang. human immunodeficency virus*) wraz z sukcesywnym wzrostem częstości izolowania wielolekoopornych prątków gruźlicy typu MDR (*ang. multi-drug resistant*) lub XDR (*ang. extremely-drug resistant*) stanowi znaczący problem epidemiologiczny. Wysoki koszt leczenia gruźlicy lekoopornej jest przyczyną stosunkowo niskiego odsetka skutecznych terapii. Stąd, pomimo trwających od stulecia profilaktycznych szczepień przeciwgruźliczych, gruźlica powraca na listę chorób nieuleczalnych (Lange et al. 2018; World Health Organization (Global Tuberculosis Programme) 2020).

Opracowanie nowych metod diagnozowania TB, nowych szczepionek przeciwgruźliczych, zarówno profilaktycznych, jak i po-ekspozycyjnych, oraz efektywniejszych i lepiej tolerowanych leków przeciwprątkowych jest wciąż niezbędne do pokonania problemu gruźlicy na świecie. Nadzieją na realizację powyższych wyzwań są badania nakierowane na lepsze wyjaśnienie mechanizmów odpornościowych chroniących przed infekcją *M.tb*, jak i wywoływanych przez sam patogen procesów patologicznych (Dheda, Barry, and Maartens 2016). Za kluczowe ogniwo w odpowiedzi odpornościowej na mykobakterie uważa się aktywność grasiczo-zależnych limfocytów T pomocniczych Th (T helper) o fenotypie Th1, które rozpoznają antygeny prątków wydzielając IFN-γ (interferon-gamma), uznawany za główną cytokinę kontrolującą rozwój *M.tb*. Najważniejszym efektem działania IFN-γ jest zwiększenie aktywności prątkobójczej makrofagów oraz nasilenie ekspresji antygenów MHC klasy II na komórkach prezentujących antygeny limfocytom T, co sprzyja rozwojowi odporności swoistej. Do ważnych cytokin nasilających wytwarzanie IFN-γ należy interleukina-18 (IL-18), której rolę i aktywność biologiczną przedstawiono w dwóch, składających się na niniejszą rozprawę doktorską, pracach przeglądowych **(Wawrocki et al. 2016; Wawrocki et al. 2017).** Cytokina ta, należąca do rodziny IL-1, wytwarzana jest przede wszystkim przez aktywowane makrofagi, ale rownież monocyty, komórki dendrytyczne, limfocyty T i B, a także keratynocyty, komórki nabłonkowe i osteoblasty. Jest ona magazynowana w syntetyzujących ją komórkach w postaci nieaktywnego biologicznie prekursora, który przekształcany jest w aktywną dojrzałą formę IL-18 dopiero po zadziałaniu enzymów, między innymi kaspazy-1. Kaspaza-1 jest białkiem wchodzącym w skład inflamasomu będącego wielobiałkowym kompleksem obecnym w cytoplazmie komórek, który dodatkowo tworzony jest przez receptory NLR (ang. *nucleotide oligomerization domain (NOD)-like receptors*) i cząsteczki adaptorowe ASC (ang. *apoptosis-associated speck-like protein*) zawierające domenę CARD (ang. *caspase activation and recruitment domain*), wiążącą i przekształcającą proenzym kaspazę-1 do formy aktywnej. Inflamasomy formowane są w odpowiedzi na pojawienie się drobnoustrojów i rozpoznanie PAMP (ang. *pathogen-associated molecular pattern*) przez receptory NLR (**Wawrocki et al. 2017).** Prekursor IL-18 może być również przekształcany na drodze alternatywnej zależnej od Fas, która prowadzi do powstania kaspazy-8 uczestniczącej w procesie powstawania aktywnej formy IL-18 (Bossaller et al. 2012). Z kolei nieaktywna biologicznie IL-18 dostająca się do środowiska pozakomórkowego z obumierających komórek może zostać aktywowana przez proteazy neutrofilowe, np. proteazę 3 (Kaplanski 2018).

Aktywacja komórki przez IL-18 wymaga powierzchniowej ekspresji dwóch podjednostek receptora IL-18R - IL-18Rα i IL-18Rβ. Podjednostka IL-18Rα wiąże IL-18, natomiast podjednostka IL-18Rβ, po związaniu IL-18 przez IL-18Rα, oddziela się od niej i przemieszcza do cytoplazmy komórki indukując wewnątrzkomórkową kaskadę sygnałową (Booker and Grattan 2014; Dinarello et al. 2013; Klekotka, Yang, and Yokoyama 2010). Prozapalna aktywność IL-18 jest równoważona przez konstytutywnie wytwarzane białko IL-18BP (IL-18 binding protein), które po związaniu IL-18 uniemożliwia interakcję cytokiny z IL-18Rα prowadząc do zahamowania wzbudzanego przez nią efektu biologicznego. Utrzymywanie określonego stosunku ilościowego między IL-18 a białkiem ją wiążącym uznawane jest za kluczowy mechanizm w utrzymywaniu stanu homeostazy i zapobieganiu reakcjom immunologicznym o charakterze patologicznym. IL-18BP wykazuje również wysokie powinowactwo do innej cytokiny, a mianowicie do IL-37, która jest inhibitorem reakcji zapalnej. IL-37, ze względu na podobieństwo do IL-18, może oddziaływać z receptorem IL-18R. Po związaniu z IL-18BP, a następnie związaniu z IL-18Rβ, IL-37 hamuje aktywność prozapalną IL-18, wzmacniając antagonistyczną aktywność IL-18BP.

Kluczową rolą IL-18 w odpowiedzi odpornościowej jest regulacja aktywności przeciwprątkowej makrofagów. Stymulacja produkcji IFN-γ przez IL-18 prowadzi do syntezy reaktywnych form azotu uczestniczących w wewnątrzkomórkowym zabijaniu prątków w trakcie procesu fagocytozy (Dinarello and Fantuzzi 2003). Funkcjonując jako komórki zdolne do pochłaniania i zabijania wirulentnych prątków, a także jako komórki prezentujące prątkowe antygeny limfocytom T, makrofagi stanowią centralne ogniwo w rozwoju zarówno wrodzonej, jak i nabytej przeciwgruźliczej odporności protekcyjnej (Wlodarczyk et al. 2014). Gen kodujący ludzką IL-18 znajduje się na chromosomie 11, w pozycji 11q22.2-22.3. Gen ten składa się z sześciu eksonów i pięciu intronów; charakteryzuje go też duża polimorficzność regionu promotorowego. Cechą genu kodującego IL-18 jest obecność dwóch fragmentów o charakterze promotorowym – pierwszego w rejonie poprzedzającym gen od strony 5’ eksonu 1 i drugiego, zlokalizowanego w obrębie intronu 1. Pierwszy region promotorowy cechuje się stałą aktywnością, podczas gdy drugi składa się z sekwencji wiążących czynniki o charakterze regulatorowym, takie jak NF-κB, AP1, Sp-1 oraz PU.1, których pobudzenie zależy od środowiska cytokinowego. Najnowsze doniesienia naukowe wskazują na wystepowanie polimorfizmu zasad w obrębie sekwencji regulatorowych genu kodującego IL-18, które mogą wpływać na wiązanie czynników transkrypcyjnych, co skutkować może zaburzeniem

ekspresji i aktywności IL-18. Zidentyfikowano także obecność swoistych populacyjnie różnic na poziomie pojedynczych nukleotydów w obrębie eksonu 1 (Dinarello et al. 2013; Giedraitis et al. 2001; Pawlik et al. 2009; Taheri et al. 2012).

Epidemiologiczne znaczenie gruźlicy w pełni przekonuje o konieczności podejmowania wysiłków zmierzających do opracowania nowych mierzalnych wskaźników rozwijanej odporności protekcyjnej, mogących przyśpieszyć oraz udoskonalić diagnozowanie aktywnej i utajonej gruźlicy. **Duże znaczenie IL-18 w rozwoju odporności przeciwgruźliczej uzasadnia podjęcie w prezentowanej rozprawie doktorskiej badań będących próbą poszszerzenia wiedzy o molekularnych i funkcjonalnych wyznacznikach udziału tej cytokiny w inicjowanych przez *M.tb* procesach odpornościowych i zweryfikowaniu sugestii o potencjalnej wartości oznaczania IL-18 i białek funkcjonalnego kompleksu tej cytokiny (IL-18BP, IL-37, IFN-γ) w diagnozowaniu gruźlicy aktywnej i latentnej.**

## Cel pracy

Celem rozprawy doktorskiej było zweryfikowanie hipotezy, że IL-18 wraz z białkiem wiążącym tę cytokinę IL-18BP, a także cytokiny współdziałające z IL-18, IL-37 i IFN-γ, to ważne ogniwa odpowiedzi odpornościowej na mykobakterie, mogące korelować ze statusem latentnego lub aktywnego zakażenia *M.tb*. Poprzez równoczesne oznaczenie poziomu IL-18 i jej naturalnego inhibitora - białka IL-18BP, wykonane badania są pierwszymi, w których zbadano udział wolnej IL-18 w aktywnej lub latentnej formie gruźlicy. Założenie udziału IL-18 w wywoływanych przez *M.tb* reakcjach odpornościowych zostało poddane weryfikacji na poziomie molekularnym i funkcjonalnym poprzez realizowane cele szczegółowe obejmujące:

1) **analizę częstości występowania polimorfizmów regionu promotorowego genu kodującego IL-18 w pozycjach IL-18/-607C/A (rs1946518) i IL-18/-137G/C (rs187238) oraz ocenę ich związku z ryzykiem zachorowania na gruźlicę w populacji polskiej;**
2) **zbadanie występowania zależności między ekspresją mRNA IL-18, IL-18BP, IL-18R, IL-37 i IFN-γ w komórkach jednojądrzastych pełnej krwi obwodowej oraz aktywną lub latentną formą gruźlicy;**
3) **ocenę przydatności oznaczania surowiczego poziomu IL-18 (w formie wolnej oraz całkowitej - związanej z IL-18BP), IL-18BP, IFN-γ, IL-37 oraz chemokiny IP-10 (białka indukującego IFN-γ), a także ilości tych mediatorów wydzielanych przez stymulowane antygenami *M.tb* leukocyty pełnej krwi obwodowej dawców w diagnozowaniu aktywnejgruźlicy płuc, utajonego zakażenia prątkami gruźlicy oraz nieprątkowego zapalenia płuc.**

## Metodyka badań

Badaniami realizowanymi w ramach projektu Preludium 10 finansowanego ze środków Narodowego Centrum Nauki, których wyniki wykorzystano w przygotowaniu rozprawy doktorskiej, objęto 238 pełnoletnich ochotników obu płci zamieszkujących województwo łódzkie, w tym 1) pacjentów z czynną gruźlicą płuc, 2) pacjentów z ostrym nieprątkowym zapaleniem płuc z wykluczonym zakażeniem *M.tb*, 3) zdrowych ochotników nigdy nie chorujących na gruźlicę, 3a) latentnie zakażonych lub 3b) niezakażonych prątkami gruźlicy. Pacjenci zaklasyfikowani zostali do odpowiednich grup badanych na podstawie wyników badań przeprowadzonch przez pulmonologów w Wojewódzkim Zespole Zakładów Opieki Zdrowotnej Centrum Leczenia Chorób Płuc i Rehabilitacji w Łodzi, natomiast osoby zdrowe zostały zrekrutowane spośród doktorantów i pracowników Wydziału BiOŚ UŁ. Wszyscy ochotnicy (pacjenci i osoby zdrowe) wyrazili pisemną zgodę na udział w badaniu przed jego rozpoczęciem. Na przeprowadzenie doświadczeń z wykorzystaniem materiału ludzkiego uzyskano zgodę Komisji do spraw bioetyki badań naukowych UŁ (uchwała nr 17/KBBN-UŁ/II/2016).

Badania obejmowały:

1. izolację genomowego DNA z komórek krwi obwodowej dawców z wykorzystaniem zestawu QIAMP DNA Mini Kit (QIAGEN, Niemcy) oraz ocenę stężenia i czystości uzyskanych izolatów DNA metodą spektrofotometryczną (Nano-Drop)
2. ocenę polimorfizmu regionu promotorowego genu kodującego IL-18 w pozycjach IL-18/-607C/A (rs1946518) i IL-18/-137G/C (rs187238) techniką PCR (Giedraitis et al. 2001).
3. izolację RNA z leukocytów krwi obwodowej ochotników z zastosowaniem zestawu QIAamp RNA Blood Mini Kit (QIAGEN, Niemcy), ocenę stężenia RNA metodą spektrofotometryczną i stopnia jego degradacji w elektroforezie w żelu agarozowym lub kapilarnej (Bioanalyzer 2100, Centrum Badawcze Bionanopark w Łodzi)
4. syntezę cDNA metodą odwrotnej transkrypcji z wykorzystaniem zestawu iScriptTM cDNA Synthesis Kit (Bio-Rad, USA)
5. ocenę stabilności ekspresji 14 genów referencyjnych (ACTB, RPL13A, B2M, RPLP0, G6PD, RPS18,GAPDH, TBP, GUSB, TFRC, HMBS, YWHAZ, HPRT1, PGK1, IPO) w ludzkich leukocytach z użyciem Reference Gene qPCR Panel (Bio-Rad, USA) zakończoną wyborem dwóch najbardziej stabilnych genów

(GAPDH i HPRT1) wykorzystanych do analizy ekspresji mRNA IL-18, IL-18BP, IL-18R, IFN-γ i IL-37 metodą porównawczą (ΔΔCt).

6. ocenę ekspresji mRNA IL-18, IL-18BP, IL-18R, IFN-γ i IL-37 techniką qPCR
7. uzyskanie surowicy z krwi obwodowej ochotników
8. wykonanie testu interferonowego QuantiFERON-TB Gold Plus (QIAGEN, Niemcy) opartego na mierzeniu produkcji IFN-γ w stymulowanych specyficznymi antygenami *M.tb* hodowlach pełnej krwi obwodowej ochotników
9. immunoenzymatyczną ocenę poziomu IL-18, IL-18BP, IFN-γ, IL-37 i IP-10 w uzyskanych surowicach oraz w supernatantach hodowli leukocytów pełnej krwi po stymulacji specyficznymi antygenami *M.tb*, z wykorzystaniem zestawów DuoSet ELISA (R&D). Poziom wolnej IL-18 (nie związanej z IL-18BP) obliczono na podstawie wyznaczonych stężeń całkowitej IL-18 i wiążącego ją białka, korzystając z prawa działania mas i równania opisanego przez Migliorini i współpracowników (Migliorini et al. 2010):

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$x$ – wolna IL-18

$a$ = stała dysocjacji Kd wynosząca 0,4nM

$b$ = [IL-18BP] - [IL-18] + Kd

$c$ = -Kd • [IL-18]

## Wyniki

Odnotowywana w literaturze zróżnicowana podatność na zachorowanie na gruźlicę w różnych populacjach świata stała się podstawą do podjęcia w niniejszej rozprawie doktorskiej badań mających na celu sprawdzenie, czy polimorfizmy genu kodującego IL-18 mogą stanowić czynnik ryzyka zachorowania na gruźlicę w populacji polskiej (**Wawrocki et al. 2019a).** Częstość występowania dwóch wariantów polimorficznych w obrębie promotora genu kodującego IL-18 - IL-18/-607C/A (rs1946518) i IL-18/-137 G/C (rs187238) oceniono u pacjentów chorych na gruźlicę i zdrowych ochotników. **Przeprowadzone badania nie wykazały występowania zależności między badanymi polimorfizmami a zachorowalnością na TB w badanej populacji (Wawrocki et al. 2019a).** Homozygotyczny genotyp G/G IL-18 (-137G/C) był najczęściej występującym wariantem w całej badanej kohorcie, który charakteryzował 47% pacjentów z gruźlicą i 48% zdrowych ochotników niezakażonych prątkami. Genotyp heterozygotyczny G/C IL-18 (-137G/C) charakteryzował się częstością 43% wśród pacjentów z TB i 42% wśród zdrowych ochotników, podczas gdy genotyp homozygotyczny C/C IL-18 (-137G/C) cechował 10% ochotników we wszystkich grupach. W zakresie polimorfizmu IL-18/-607C/A, genotyp heterozygotyczny C/A (IL-18/-607C/A) stwierdzono u 51% pacjentów z TB i 54% zdrowych ochotników; homozygotyczny C/C (IL-18/-607C/A) u 40% pacjentów z TB i 34% osób zdrowych, a homozygotyczny A/A (IL-18/-607C/A) u 9% chorych na TB i 12% zdrowych ochotników (**Wawrocki et al. 2019a).** Uzyskane wyniki pozostają w zgodzie z danymi pochodzącymi z innych krajów świata, takich jak Indie, Chiny czy Iran (Harishankar et al. 2007; Taheri et al. 2012; Zhou et al. 2015). **W przeprowadzonych badaniach nie wykazano wpływu analizowanych wariantów IL-18 na podatność na zachorowanie na gruźlicę w Polsce, ale także nie wykazano występowania zależności pomiędzy badanymi polimorfizmami a poziomem IL-18 mierzonym immunoenzymatycznie w surowicy.** Warto podkreślić, że w grupie chorych na TB zaobserwowano znamiennie wyższy poziom tej cytokiny niż w zdrowej grupie kontrolnej. Wynik taki sugeruje, że wzrost stężenia IL-18 obserwowany w aktywnej gruźlicy może nie mieć istotnego związku z wybranymi polimorfizmami regionu promotorowego genu kodującego IL-18, ale prawdopodobnie jest konsekwencją wzbudzonych przez *M.tb* procesów odpornościowych.

Biorąc pod uwagę zaobserwowany u osób chorujących na gruźlicę wzrost surowiczego stężenia IL-18, w kolejnym etapie pracy doktorskiej postanowiono poddać

to białko dokładniejszej analizie molekularnej oceniając ekspresję mRNA IL-18, IL-18BP, IL-18R oraz IL-37 oraz IFN-γ **(Wawrocki et al. 2020b).** Jako źródło RNA posłużyły leukocyty pełnej krwi obwodowej pacjentów z aktywną gruźlicą (TB), osób z latentnym zakażeniem *M.tb* (LTBI) oraz osób zdrowych, niezakażonych prątkami gruźlicy (HC). Latentne zakażenie *M.tb* zostało potwierdzone dodatnim wynikiem testu interferonowego z pełnej krwi. Po raz pierwszy wykazano **znamiennie wyższą względną ekspresję mRNA IL-18 i IL-18BP zarówno w aktywnym, jak i latentnym zakażeniu *M.tb*.** Co ciekawe, ekspresja mRNA receptora IL-18R była istotnie niższa w grupie osób z latentnym zakażeniem *M.tb* w porównaniu do grupy pacjentów chorujących na czynną gruźlicę, jak i osób zdrowych. Jednocześnie, wraz z niską ekspresją mRNA IL-18R u osób z LTBI obserwowano niską ekspresję mRNA IFN-γ, na poziomie równym ekspresji tej cytokiny u osób zdrowych bez zakażenia *M.tb*. W porównaniu do grup LTBI i HC, u pacjentów z aktywną TB zaobserwowano wyższą ekspresję mRNA IFN-γ w leukocytach. Natomiast ekspresja mRNA IL-37 pozostawała na porównywalnym poziomie w każdej z grup. Przedstawione wyniki sugerują, że **zwiększona ekspresja mRNA IL-18, zmniejszona ekspresja mRNA IL-18R i brak wzrostu ekspresji mRNA IFN-γ moga być nowym zestawem wskaźników charakteryzujących stan równowagi między odpowiedzią odpornościową gospodarza a metabolicznym uśpieniem *M.tb* w trakcie utajonego bezobjawowego zakażenia.** Zredukowana ekspresja mRNA IL-18R w latentnej infekcji pozwala rozważyć jej znaczenie jako czynnika regulującego rozwój adaptacyjnych mechanizmów odporności komórkowej, umożliwiającego długotrwałe utrzymywanie się metabolicznie wyciszonych prątków *M.tb* w zakażonych komórkach gospodarza.

Odnotowany w aktywnej gruźlicy wzrost ekspresji mRNA IL-18, IL-18BP oraz IFN-γ, skłonił w kolejnym etapie pracy doktorskiej do ilościowej analizy poziomu sekrecji tych białek i sprawdzenia ich użyteczności jako pomocniczych biomarkerów infekcji gruźliczej (**Wawrocki et al. 2019b).** Uwzględniając liczne dane literaturowe wskazujące na większą wartość diagnostyczną w badaniach przesiewowych złożonych paneli białek odpornościowych w porównaniu do pojedynczych biomarkerów, w badaniach określono stężenie oraz wzajemne stosunki ilościowe IL-18 (w formie całkowitej - związanej z IL-18BP oraz wolnej), IL-18BP, IL-37 i IFN-γ w surowicy oraz supernatantach hodowli leukocytów w pełnej krwi uzyskanych po stymulacji specyficznymi antygenami *M.tb*. Poziom wolnej IL-18 obliczono na podstawie wyznaczonych stężeń całkowitej IL-18 i wiążącego ją białka (IL-18BP), korzystając z prawa działania mas (Migliorini et al. 2010). W badaniach uwzględniono również kwantyfikację chemokiny IP-10 (CXCL10), znanej

również jako białko indukujące IFN-γ, odpowiedzialnej za przyciąganie komórek zapalnych do miejsca toczącej się infekcji. Badaniami objęto zarówno pacjentów z aktywną gruźlicą (TB), osoby z latentnym zakażeniem *M.tb* (LTBI) oraz zdrowych niezakażonych prątkami gruźlicy ochotników (HC) (**Wawrocki et al. 2019b). Uzyskane wyniki wykazały znamiennie wyższe surowicze stężenia całkowitej i wolnej IL-18, IL-18BP, IFN-γ i IP-10, zaś niższe stężenie IL-37 wśród chorych z TB w porównaniu do poziomu markerów u osób z grup LTBI lub HC.** Badania poziomu analizowanych białek wykonane w hodowlach krwi po stymulacji antygenami prątków wykazały w grupie pacjentów z TB znamiennie wyższe stężenia całkowitej IL-18 oraz IL-18BP, natomiast istotnie niższe stężenie IFN-γ, w porównaniu do poziomu tych białek odnotowanego w grupach LTBI oraz HC. Uzyskane wyniki wskazują zatem na znamiennie wyższe stężenie krążącego IFN-γ i jednoczesne obniżenie poziomu tej cytokiny w stymulowanych antygenami *M.tb* hodowlach pełnej krwi pacjentów z aktywną TB w porównaniu do osób z grupy LTBI. Różnica taka sugeruje, że aktywacja odpowiedzi przeciwgruźliczej podczas czynnej TB występuje jednocześnie ze zjawiskiem supresji immunologicznej. Nie można również wykluczyć, że w trakcie czynnej TB dochodzi do redystrybucji najbardziej reaktywnych wobec *M.tb* limfocytów T z krążenia do płuc, do miejsca toczącego się zakażenia, co sprawia, że w krążeniu pozostają specyficzne dla *M.tb* limfocyty T słabiej odpowiadające na ich antygeny. Obserwowany u chorych z TB podwyższony poziom IP-10 w surowicy przemawia za taką interpretacją, ponieważ chemokina ta odpowiada za rekrutację limfocytów Th1 i komórek NK do miejsc objętych zakażeniem. Oceniając surowiczy, jak i stymulowany antygenami *M.tb,* poziom wydzielania IL-37 nie stwierdzono istotnych różnic w grupach objętych badaniem. A zatem, jak wskazują uzyskane w pracy wyniki, **towarzysząca aktywnej TB nasilona sekrecja IL-18, IL-18BP, IFN-γ i IP-10 nie zostaje zrównoważona przez nadprodukcję IL-37, co pozwala sugerować znaczną utratę równowagi w zakresie sygnału wzbudzanego przez IL-18 podczas czynnego zakażenia *M.tb* (Wawrocki et al. 2019b).** Analiza statystyczna wykazała, że wszystkie badane w surowicy markery odpornościowe, z wyjątkiem IL-37, posiadają potencjał w rozróżnianiu aktywnej i utajonej gruźlicy. **Najlepszymi surowiczymi zestawami biomarkerów odróżniającymi aktywną TB od latentnej infekcji okazały się być pary IL-18BP~IL-37 oraz IP-10~IFN-γ (Wawrocki et al. 2019b).** Spośród białek badanych w hodowlach pełnej krwi stymulowanych antygenami *M.tb* potencjałem różnicującym charakteryzowały się wyłącznie całkowita IL-18 i IFN-γ. Wykazano ponadto, że wzajemne proporcje pomiędzy stężeniem badanych biomarkerów w surowicy oraz supernatantach hodowli stymulowanych

antygenami *M.tb* mogą stanowić równie czułe wskaźniki zakażenia prątkami gruźlicy, jak sam ich mierzalny poziom. Największą siłą dyskryminującą grupę TB od LTBI charakteryzowały się proporcje ilościowe między całkowitą IL-18/IL-18BP, całkowitą IL-18 /IL-37, całkowitą IL-18/IFN-γ oraz IL-18BP/IP-10 **(Wawrocki et al. 2019b). Uzyskane w pracy wyniki pozwalają sugerować, że ilościowa analiza surowiczych białek kompleksu IL-18/IL-18BP/IL-37 poszerzona o ocenę chemokiny IP-10 może mieć zastosowanie w projektowaniu nowych szybkich testów przesiewowych do diagnozowania gruźlicy płuc.**

W odpowiedzi na potrzebę opracowania nowych testów diagnostycznych umożliwiających różnicowanie aktywnej gruźlicy płuc, o różnym stopniu zaawansowania zmian chorobowych, od nieprątkowych postaci zapalenia płuc, w niniejszej pracy doktorskiej zadano pytanie, czy ilościowa analiza białek kompleksu IL-18/IL-18BP/IL-37 może stanowić w tym zakresie pomocnicze narzędzie diagnostyczne **(Wawrocki et al. 2020a).** W tym celu porównano surowicze oraz stymulowane specyficznymi antygenami *M.tb* stężenie IL-18 (w formie całkowitej oraz wolnej), IL-18BP, IL-37, IP-10 i IFN-γ w grupach pacjentów z zapaleniem płuc o innej niż prątkowa etiologii (CAP, ang. *community-acquired pneumonia*) oraz pacjentów z aktywną TB z łagodnymi/umiarkowanymi (M/MTB; ang. *mild/moderate*) lub zaawansowanymi (ATB; ang. *advanced TB*) zmianami w tkance płucnej. W badaniach zaobserwowano, że podczas gdy w grupie pacjentów z zaawansowaną TB podwyższonemu surowiczemu stężeniu IL-18BP towarzyszył wyższy poziom całkowitej IL-18, w grupie pacjentów z M/MTB nadprodukcji IL-18BP nie towarzyszył wzrost stężenia tej cytokiny **(Wawrocki et al. 2020a).** W konsekwencji, korelacje obserwowane między sekrecją IL-18 a wydzielaniem IFN-γ oraz IL-18BP a IFN-γ były specyficzne wyłącznie dla chorych z zaawansowaną TB, a nie dla chorych z M/MTB. Zależność między wolną IL-18 i IL-18BP odnotowano wyłącznie w grupie chorych z łagodnym/umiarkowanym zaawansowaniem zmian gruźliczych. Można postawić hipotezę, że podwyższony poziom krążącego IL-18BP był wystarczający, by przeciwdziałać wzrostowi IL-18 w grupie M/MTB, ale niewystarczający u pacjentów z ATB, co prawdopodobnie wynika z bardziej intensywnej sekrecji IL-18 u chorych z zaawansowanym uszkodzeniem płuc. Wykazana w pracy **nadprodukcja IL-18, nie zrównoważona wystarczająco przez IL-18BP, wskazuje na potencjalną rolę IL-18 w patomechanizmie gruźlicy płuc.** Warto wspomnieć, że IL-18BP występuje w ustroju w różnych izoformach o różnym powinowactwie do IL-18. Izoformy a (IL-18BPa) i c (IL-18BPc) tego białka skutecznie neutralizują aktywność IL-18, podczas gdy izoformy

IL-18BPb i IL-18BPd nie mają zdolności wiązania i neutralizowania tej cytokiny (Kim et al. 2000). Jest więc prawdopodobne, że jednoczesny wzrost stężeń IL-18BP i IL-18 obserwowany w grupie ATB, może wynikać, przynajmniej częściowo, z preferencyjnego wydzielania niefunkcjonalnych izoform IL-18BP. Ta obserwacja może wskazywać na IL-18BP jako potencjalny cel w nowych przeciwgruźliczych podejściach terapeutycznych. W szeregu badań wykazano bowiem możliwość zastosowania IL-18BP w leczeniu chorób charakteryzujących się zaburzeniem stosunku IL-18/IL-18BP w płynach ustrojowych. Podawanie egzogennej IL-18BP jest obiecującą strategią terapeutyczną m.in. w leczeniu łuszczycy, reumatoidalnego zapalenia stawów czy miażdżycy tętnic (Banda et al. 2003; Chiossone et al. 2012).

Podsumowując, w odpowiedzi na potrzebę wytypowania biomarkerów odpornościowych pozwalających na **odróżnienie aktywnej gruźlicy płuc o różnym stopniu zaawansowania zmian chorobowych od nieprątkowych postaci zapalenia płuc**, przeprowadzono wieloparametryczną analizę statystyczną, której wyniki wykazały, że **największy potencjał diagnostyczny w tym zakresie przedstawiała jednoczesna ocena dwóch surowiczych białek - IL-18BP i IP-10 oraz ich wzajemny stosunek ilościowy (IL-18BP/IP-10) (Wawrocki et al. 2020a).** Ponadto na podstawie uzyskanych danych ustalono również, że iloraz surowiczego poziomu wolnej IL-18 i IP-10 (wolna IL-18/IP-10) oraz wolnej IL-18 i IL-18BP (wolna IL-18/IL-18BP) posiadał najlepszą wartość diagnostyczną w różnicowaniu pacjentów z M/MTB od chorych z CAP, natomiast w diagnostyce różnicowej między ATB a CAP najwyższą mocą różnicującą cechował się, mierzony w surowicy, wzajemny stosunek całkowitej IL-18/IFN-γ oraz IL-18BP/IFN-γ. Mimo wykazanej w pracy możliwości wykorzystania ilościowej oceny IL-18BP, IP-10 oraz IL-18BP/IP-10 w różnicowej diagnostyce TB i CAP, nie udało się znaleźć wśród badanych białek takich markerów, które pozwoliłyby na różnicowanie gruźlicy z zaawansowanymi i łagodnymi/umiarkowanymi zmianami w płucach. Nie stwierdzono również różnic w stężeniach badanych białek u pacjentów z gruźlicą płuc potwierdzoną bakteriologicznie oraz tych z ujemnym wynikiem hodowli *M.tb*. Wykazano ponadto, że ilościowa ocena krążących w surowicy biomarkerów odpornościowych jest lepszym podejściem diagnostycznym niż ocena ich stężenia w supernatantach hodowli pełnej krwi stymulowanych specyficznymi antygenami *M.tb*. W przeciwieństwie bowiem do surowicy w supernatantach hodowli prowadzonych dla porównywanych grup osób objętych badaniami nie obserwowano znaczących różnic w poziomach badanych białek.

## Podsumowanie wyników

W badaniach wykazano:

1. brak zależności pomiędzy polimorfizmem regionu promotorowego genu kodującego IL-18 w pozycjach IL-18/-607C/A (rs1946518) i IL-18/-137G/C (rs187238) a osobniczą podatnością na zachorowanie na gruźlicę w populacji polskiej;
2. brak korelacji pomiędzy polimorfizmem regionu promotorowego genu kodującego IL-18 w pozycjach IL-18/-607C/A (rs1946518) i IL-18/-137G/C (rs187238) a surowiczym poziomem IL-18 w badanej kohorcie;
3. znamiennie wyższą ekspresję mRNA IL-18 i IL-18BP w leukocytach pacjentów z aktywną TB, jak i zdrowych osób z latentnym zakażeniem *M.tb,* w porównaniu do zdrowych ochotników niezakażonych prątkami gruźlicy;
4. znamiennie wyższą ekspresje mRNA IFN-γ w leukocytach pacjentów z aktywną TB w porównaniu do osób z latentnym zakażeniem *M.tb* lub zdrowych niezakażonych prątkami ochotników;
5. znamiennie niższą ekspresję mRNA IL-18R w leukocytach zdrowych osób z latentnym zakażeniem *M.tb* w porównaniu do pacjentów z aktywną TB lub zdrowych niezakażonych prątkami ochotników;
6. brak różnic w ekspresji mRNA IL-37 w leukocytach pacjentów z aktywną TB, osób z latentnym zakażeniem *M.tb* i zdrowych niezakażonych prątkami ochotników;
7. znamiennie wyższe surowicze stężenie całkowitej i wolnej IL-18, IL-18BP, IFN-γ i IP-10, zaś obniżenie poziomu IL-37 u chorych z TB w porównaniu z osobami z latentnym zakażeniem *M.tb* i zdrowych niezakażonych prątkami ochotników;
8. znamiennie wyższe stężenie całkowitej IL-18 oraz IL-18BP i istotnie niższe stężenie IFN-γ w hodowlach pełnej krwi stymulowanych specyficznymi antygenami *M.tb* u pacjentów z TB w porównaniu do osób z latentnym zakażeniem *M.tb* i zdrowych niezakażonych prątkami ochotników;
9. najlepszymi surowiczymi zestawami biomarkerów odróżniającymi aktywną TB od latentnej infekcji *M.tb* okazały się być IL-18BP~IL-37 oraz IP-10~IFN-γ;
10. wzajemne proporcje pomiędzy stężeniem biomarkerów odpornościowych w surowicach lub supernatantach hodowli pełnej krwi stymulowanych antygenami *M.tb* mogą stanowić czułe wskaźniki zakażenia prątkami gruźlicy, jak ich mierzalny poziom;

11. podwyższone surowicze stężenie IL-18BP i towarzyszący mu wyższy poziom całkowitej IL-18 u pacjentów z zaawansowaną TB (ATB) oraz nadprodukcję IL-18BP, której nie towarzyszył wzrost stężenia tej cytokiny u pacjentów z łagodnym/umiarkowanym zaawansowaniem zmian gruźliczych (M/MTB);
12. brak różnic w poziomie IL-18 (w formie wolnej, jak i związanej z IL-18BP), IL-18BP, IFN-γ, IL-37 i IP-10 w surowicy lub w stymulowanych antygenami prątków hodowlach pełnej krwi u pacjentów z gruźlicą płuc potwierdzoną bakteriologicznie oraz tych z ujemnym wynikiem hodowli *M.tb*;
13. iloraz surowiczego poziomu wolnej IL-18 i IP-10 (wolna IL-18/IP-10) oraz wolnej IL-18 i IL-18BP (wolna IL-18/IL-18BP) posiadał najlepszą wartość diagnostyczną w różnicowaniu pacjentów z M/MTB od chorych z nieprątkowym zapaleniem płuc (CAP), natomiast w diagnostyce różnicowej między ATB a CAP najwyższą mocą dyskryminującą cechował się wzajemny stosunek całkowitej IL-18/IFN-γ oraz IL-18BP/IFN-γ w surowicy;
14. największy potencjał dyskryminujący jednoczesnej oceny dwóch surowiczych białek - IL-18BP i IP-10 oraz ich wzajemnej proporcji ilościowej (IL-18BP/IP-10) umożliwiający odróżnienie aktywnej gruźlicy płuc od nieprątkowego zapalenia płuc.

**Wnioski końcowe:**

I. **Wzrost ekspresji mRNA IL-18, zmniejszona ekspresja mRNA IL-18R i brak wzrostu ekspresji mRNA IFN-γ mogą być nowym zestawem wskaźników charakteryzujących stan równowagi między odpowiedzią odpornościową gospodarza a metabolicznym uśpieniem *M.tb* w trakcie utajonego bezobjawowego zakażenia.**

II. **Nadprodukcja IL-18, nie zrównoważona przez IL-18BP, w aktywnej gruźlicy płuc, może wskazywać na potencjalną rolę IL-18 w patomechanizmie tej choroby.**

III. **Ilościowa analiza surowiczych białek kompleksu IL-18/IL-18BP/IL-37 poszerzona o ocenę poziomu chemokiny IP-10, potencjalnie może mieć zastosowanie w diagnostyce aktywnego lub utajonego zakażenia *M.tb* i różnicowaniu nieprątkowego zapalenia płuc.**

# Streszczenie

Gruźlica (TB), wywoływana przez prątki *Mycobacterium tuberculosis (M.tb*), od lat pozostaje światowym problemem zdrowotnym, uznawanym za chorobę zakaźną o jednej z najwyższej śmiertelności, pomimo prowadzonych na szeroką skalę profilaktycznych szczepień przeciwgruźliczych. Według najnowszych danych Światowej Organizacji Zdrowia 1/4 światowej populacji jest zakażona *M.tb.* Zakażenie to może mieć bezobjawową postać utajoną (latentną), które jednak u co najmniej 10% zakażonych osób rozwija się w aktywną postać choroby. Epidemiologiczne znaczenie gruźlicy uzasadnia konieczność podejmowania wysiłków zmierzających do opracowania nowych mierzalnych wskaźników rozwijanej odporności protekcyjnej, mogących przyśpieszyć oraz udoskonalić diagnozowanie aktywnej i utajonej gruźlicy. Nadzieją na realizację powyższych wyzwań są badania nakierowane na lepsze wyjaśnienie mechanizmów odpornościowych chroniących przed infekcją *M.tb*, jak i wywoływanych przez sam patogen procesów patologicznych.

Za kluczowy element w odpowiedzi odpornościowej na mykobakterie uważa się aktywność grasiczo-zależnych limfocytów T pomocniczych Th (T helper) o fenotypie Th1, które rozpoznają antygeny prątków wydzielając IFN-γ (*interferon-gamma*), uznawany za główną cytokinę kontrolującą rozwój *M.tb*. Do ważnych cytokin nasilających wytwarzanie IFN-γ należy interleukina-18 (IL-18), należąca do rodziny IL-1. Celem rozprawy doktorskiej było zweryfikowanie hipotezy, że IL-18 wraz z rozpoznającym ją receptorem (IL-18R) i wiążącym białkiem IL-18BP (*IL-18 binding protein*), a także cytokiny współdziałające z IL-18, IL-37 i IFN-γ, to ważne ogniwa odpowiedzi odpornościowej na mykobakterie, mogące korelować ze statusem latentnego lub aktywnego zakażenia *M.tb*. Duże znaczenie IL-18 w rozwoju odporności przeciwgruźliczej uzasadnia podjęcie w prezentowanej rozprawie doktorskiej badań będących próbą rozszerzenia wiedzy o molekularnych i funkcjonalnych wyznacznikach udziału tej cytokiny w inicjowanych przez *M.tb* procesach odpornościowych i zweryfikowaniu sugestii o potencjalnej wartości oznaczania IL-18 i białek funkcjonalnego kompleksu tej cytokiny (IL-18BP, IL-37, IFN-γ) w diagnozowaniu gruźlicy aktywnej i latentnej.

Wyniki przeprowadzonych badań wykazały, iż wzrost ekspresji mRNA IL-18, zmniejszona ekspresja mRNA IL-18R i brak wzrostu ekspresji mRNA IFN-γ mogą być nowym zestawem wskaźników charakteryzujących stan równowagi między odpowiedzią odpornościową gospodarza a metabolicznym uśpieniem *M.tb* w trakcie utajonego bezobjawowego zakażenia. Jednocześnie stwierdzono, iż nadprodukcja IL-18,

nie zrównoważona przez IL-18BP, w aktywnej gruźlicy płuc, może wskazywać na potencjalną rolę IL-18 w patomechanizmie tej choroby. Natomiast ilościowa analiza surowiczych białek kompleksu IL-18/IL-18BP/IL-37 poszerzona o ocenę poziomu chemokiny IP-10, potencjalnie może mieć zastosowanie w diagnostyce aktywnego lub utajonego zakażenia *M.tb* i różnicowaniu nieprątkowego zapalenia płuc.

# Summary

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M.tb*), has been a global health problem for years, considered to be an infectious disease with one of the highest mortality rates, despite the large-scale prophylactic vaccination against tuberculosis. According to the World Health Organization's latest data, 1/4 of the world's population is infected with *M.tb*. This infection may be asymptomatic (latent), but at least 10% of infected people develop the active disease. The epidemiological significance of TB justifies the necessity to create new measurable indicators of developed protective immunity, which may accelerate and improve the diagnosis of active and latent tuberculosis. The hope for meeting the above challenges is research to understand better the immune mechanisms that protect against *M.tb* infection and pathological processes caused by the pathogen itself.

Thymic-dependent T helper lymphocytes with a Th1 phenotype activity recognize mycobacterial antigens and secretes IFN-γ (interferon-gamma), which is considered the main cytokine controlling the development of *M.tb* infection, is regarded as a crucial element in the immune response to mycobacteria. An important cytokine enhancing IFN-γ production is interleukin-18 (IL-18), a member of the IL-1 family. The doctoral dissertation aimed to verify the hypothesis that IL-18, with IL-18 receptor (IL-18R) and the protein binding this cytokine IL-18BP and cytokines interacting with IL-18, IL-37, and IFN-γ are essential links in the immune response to mycobacteria that may correlate with the status of latent or active *M.tb* infection. The high importance of IL-18 in the development of anti-TB immunity justifies the undertaking of research in the presented doctoral dissertation in an attempt to broaden the knowledge about the molecular and functional determinants of the participation of this cytokine in immune processes initiated by *M.tb* and to verify the suggestions about the potential value of determining IL-18 and proteins of the functional complex of this cytokine (IL-18BP, IL-37, IFN-γ) in the diagnosis of active tuberculosis and latent.

The results of the conducted studies have shown that the increase in IL-18 mRNA expression, decreased IL-18R mRNA expression, and the lack of increase in IFN-γ mRNA expression may be a new set of indicators characterizing the state of equilibrium between the host's immune response and metabolically inactive *M.tb* during latent infection. At the same time, it was found that the overproduction of IL-18, not balanced by IL-18BP, in active pulmonary tuberculosis, may indicate a potential role of IL-18 in the pathomechanism of this disease. On the other hand, the quantitative analysis of the serum proteins of the IL-18 /

IL-18BP / IL-37 complex, extended to the assessment of the chemokine IP-10 level, could potentially be used in the diagnosis of active or latent *M.tb* infection and the differentiation of non-tuberculous pneumonia.

# Literatura


Banda, Nirmal K et al. 2003. “Mechanisms of Inhibition of Collagen-Induced Arthritis by Murine IL-18 Binding Protein.” *The Journal of Immunology* 170(4): 2100–2105. http://www.ncbi.nlm.nih.gov/pubmed/12574381 (April 12, 2019).

Booker, Chris S., and David R. Grattan. 2014. “Identification of a Truncated Splice Variant of IL-18 Receptor Alpha in the Human and Rat, with Evidence of Wider Evolutionary Conservation.” *PeerJ* 2014(1). /pmc/articles/PMC4168765/?report=abstract (October 26, 2020).

Bossaller, Lukas et al. 2012. “Cutting Edge: FAS (CD95) Mediates Noncanonical IL-1β and IL-18 Maturation via Caspase-8 in an RIP3-Independent Manner.” *The Journal of Immunology* 189(12): 5508–12. /pmc/articles/PMC3518757/?report=abstract (January 20, 2021).

Chiossone, Laura et al. 2012. “Protection from Inflammatory Organ Damage in a Murine Model of Hemophagocytic Lymphohistiocytosis Using Treatment with IL-18 Binding Protein.” *Frontiers in Immunology* 3(August 8, 2012).

Dheda, Keertan, Clifton E. Barry, and Gary Maartens. 2016. “Tuberculosis.” *The Lancet* 387(10024): 1211–26. https://pubmed.ncbi.nlm.nih.gov/26377143/ (October 26, 2020).

Dinarello, Charles A., and Giamila Fantuzzi. 2003. “Interleukin-18 and Host Defense against Infection.” In *Journal of Infectious Diseases*, J Infect Dis. https://pubmed.ncbi.nlm.nih.gov/12792854/ (October 26, 2020).

Dinarello, Charles A., Daniela Novick, Soohyun Kim, and Gilles Kaplanski. 2013. “Interleukin-18 and IL-18 Binding Protein.” *Frontiers in Immunology* 4: 289. http://www.ncbi.nlm.nih.gov/pubmed/24115947 (March 4, 2019).

Fol, Marek et al. 2011. “Szczepienia Przeciwprątkowe- BCG i Co Dalej?” *Postepy Higieny i Medycyny Doswiadczalnej* 65: 93–103. https://pubmed.ncbi.nlm.nih.gov/21357997/ (October 29, 2020).

Furin, Jennifer, Helen Cox, and Madhukar Pai. 2019. “Tuberculosis.” *The Lancet* 393(10181): 1642–56. https://pubmed.ncbi.nlm.nih.gov/30904262/ (October 26, 2020).

Giedraitis, Vilmantas, Bing He, Wen-Xin Huang, and Jan Hillert. 2001. “Cloning and Mutation Analysis of the Human IL-18 Promoter: A Possible Role of Polymorphisms in Expression Regulation.” *Journal of Neuroimmunology* 112(1–2): 146–52. https://www.sciencedirect.com/science/article/pii/S0165572800004070 (March 1, 2019).

Harishankar, M. et al. 2007. “Promoter Polymorphism of IL-18 Gene in Pulmonary Tuberculosis in South Indian Population.” *International Journal of Immunogenetics* 34(5): 317–20. http://doi.wiley.com/10.1111/j.1744-313X.2007.00714.x (March 1, 2019).

Kaplanski, Gilles. 2018. “Interleukin-18: Biological Properties and Role in Disease Pathogenesis.” *Immunological Reviews* 281(1): 138–53. https://pubmed.ncbi.nlm.nih.gov/29247988/ (October 26, 2020).

Kim, Soo Hyun et al. 2000. “Structural Requirements of Six Naturally Occurring Isoforms of the IL-18 Binding Protein to Inhibit IL-18.” *Proceedings of the National Academy of Sciences of the United States of America* 97(3): 1190–95. https://pubmed.ncbi.nlm.nih.gov/10655506/ (January 3, 2021).

Klekotka, Paul A., Liping Yang, and Wayne M. Yokoyama. 2010. “Contrasting Roles of the IL-1 and IL-18 Receptors in MyD88-Dependent Contact Hypersensitivity.” *Journal of Investigative Dermatology* 130(1): 184–91. /pmc/articles/PMC2795006/?report=abstract (October 26, 2020).

Lange, Christoph et al. 2018. “Drug-Resistant Tuberculosis: An Update on Disease Burden, Diagnosis and Treatment.” *Respirology* 23(7): 656–73. https://pubmed.ncbi.nlm.nih.gov/29641838/ (October 26, 2020).

Migliorini, Paola et al. 2010. “Serum and Urinary Levels of IL-18 and Its Inhibitor IL-18BP in Systemic Lupus Erythematosus.” *European cytokine network* 21(4): 264–71. http://www.ncbi.nlm.nih.gov/pubmed/21126942 (April 12, 2019).

Nathavitharana, Ruvandhi R., and Jon S. Friedland. 2015. “A Tale of Two Global Emergencies: Tuberculosis Control Efforts Can Learn from the Ebola Outbreak.” *European Respiratory Journal* 46(2): 293–96. https://pubmed.ncbi.nlm.nih.gov/26232473/ (October 8, 2020).

Pawlik, A. et al. 2009. “Interleukin-18 Gene ( *IL18* ) Promoter Polymorphisms in Patients with Rheumatoid Arthritis.” *Scandinavian Journal of Rheumatology* 38(3): 159–65. http://www.ncbi.nlm.nih.gov/pubmed/19229765 (March 2, 2019).

PZH. 2020a. “Kiedy Wprowadzono w Polsce Szczepienia BCG? - Szczepienia.Info.” https://szczepienia.pzh.gov.pl/faq/kiedy-wprowadzono-w-polsce-szczepienia-bcg/ (October 29, 2020).

PZH. 2020b. “Program Szczepień Ochronnych w 2020 Roku - Szczepienia.Info.” https://szczepienia.pzh.gov.pl/kalendarz-szczepien-2020/ (October 29, 2020).

Taheri, M. et al. 2012. “Lack of Association between Interleukin-18 –607 C/A Gene Polymorphism and Pulmonary Tuberculosis in Zahedan, Southeast Iran.” *Prague Medical Report* 113(1): 16–22. http://www.ncbi.nlm.nih.gov/pubmed/22373801 (March 1, 2019).

Wlodarczyk, Marcin et al. 2014. “Interferon-Gamma Assay in Combination with Tuberculin Skin Test Are Insufficient for the Diagnosis of Culture-Negative Pulmonary Tuberculosis.” *PLoS ONE* 9(9).

World Health Organisation. 2018. *Latent Tuberculosis Infection Updated and Consolidated Guidelines for Programmatic Management*. https://apps.who.int/iris/bitstream/handle/10665/260233/9789241550239-eng.pdf (April 25, 2020).

World Health Organization (Global Tuberculosis Programme). 2020. World Health Organization *Global Tuberculosis Report 2020*. http://apps.who.int/bookorders. (January 3, 2021).

Zhou, Chao et al. 2015. "The -137G/C Single Nucleotide Polymorphism in IL-18 Gene Promoter Contributes to Tuberculosis Susceptibility in Chinese Han Population." *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 36: 376–80. https://linkinghub.elsevier.com/retrieve/pii/S1567134815300149 (March 1, 2019).

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Minireview

# Interleukin 18 (IL-18) as a target for immune intervention*

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**Interleukin 18 (IL-18) is a pleiotropic cytokine involved in the regulation of innate and acquired immune response. In the milieu of IL-12 or IL-15, IL-18 is a potent inducer of IFN-gamma in natural killer (NK) cells and CD4 T helper (Th) 1 lymphocytes. However, IL-18 also modulates Th2 and Th17 cell responses, as well as the activity of CD8 cytotoxic cells and neutrophils, in a host microenvironment-dependent manner. It is produced by various hematopoietic and nonhematopoietic cells, including dendritic cells and macrophages. In an organism, bioactivity of the cytokine depends on the intensity of IL-18 production, the level of its natural inhibitory protein — IL-18BP (IL-18 binding protein) and the surface expression of IL-18 receptors (IL-18R) on the responding cells. This review summarizes the biology of the IL-18/IL-18BP/IL-18R system and its role in the host defense against infections. The prospects for IL-18 application in immunotherapeutic or prophylactic interventions in infectious and non-infectious diseases are discussed.**

**Key words**: interleukin-18 (IL-18), IL-18 receptor, IL-18 binding protein

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## INTERLEUKIN-18

Interleukin 18 (IL-18) was first described in the serum of mice inoculated intraperitoneally with endotoxin and was called the "IFN-gamma inducing factor" (Nakamura *et al.,* 1989). The name was changed to IL-18 after isolation of this cytokine from the liver extracts of mice treated with *Propionibacterium acnes* and subsequently challenged with lipopolysacharide, after molecular cloning (Okamura *et al.,* 1995). Although originally described as a factor capable of inducing IFN-gamma production by murine splenocytes, the effector role of IL-18 rapidly expanded. IL-18 is currently classified as one of the members of the IL-1 cytokine superfamily- that acts as an important regulator of innate and acquired immune responses (Garcie *et al.,* 2003; Dinarello *et al.,* 2013). This cytokine is a potent activator of polarized T helper 1 (Th1) cells for IFN-gamma production and lymphocyte proliferation (Lebel-Binay *et al.,* 2000). Some studies have shown a functionally pleiotropic and complex functioning of IL-18, depending on the host environment. This cytokine plays effector and regulatory roles in a variety of early inflammatory responses. It is also expressed at the sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in the context of numerous infectious diseases (Lebel-Binay *et al.,* 2000; Diakowska *et al.,* 2006; Kinjo *et al.,* 2002; Fabbi *et al.,* 2015).

## THE PRODUCTION AND ACTIVATION OF IL-18

In the body, IL-18 is constitutively expressed by several cell types, including macrophages, Kupffer cells, keratinocytes, osteoblasts, adrenal cortex cells, intestinal epithelial cells, microglial cells and synovial fibroblasts (Garcie *et al.,* 2003). This cytokine is produced by activated immune cells, dendritic cells, monocytes and macrophages, T and B lymphocytes, natural killer cells (NK) and neutrophils. IL-18 is produced as a 24 kDa inactive precursor (pro IL-18) lacking a signal peptide required for secretion (Okamura *et al.,* 1995). In order to be activated, it must be processed by the intracellular cysteine protease caspase-1, which cleaves the precursor into an active mature molecule of 17 200 Da (Dinarello *et al.,* 2013; Wei *et al.,* 2014). Cleavage of pro IL-18 into mature IL-18 allows this molecule to be released from the cell, although a significant amount of the IL-18 precursor remains unprocessed inside the cell. A signal, which is supplied by IL-18 to the interior of the cell, needs binding of the mature cytokine to its ligand, which is the IL-18 receptor alpha chain (IL-18Rα). However, the low affinity of binding between IL-18 and IL-18Rα prevents initialization of the signal transduction pathway and immune cell activation (Schneider *et al.,* 2010). Full activation of cells by IL-18 requires interaction between the interleukin IL-18Rα receptor and the IL-18 beta chain co-receptor (IL-18Rβ). This complex is functionally and structurally similar to other members of the IL-1 family, with the IL-1RAcP co-receptor (IL-1 receptor accessory protein). The cytoplasmic fragment of the IL-18 receptor and other receptors of the IL-1 family have a TIR domain (Toll IL-1 receptor), belonging to the Toll-like (TLR) receptors. The activation of IL-18 results in a cascade of reactions in which the Toll-IL-1 receptor (TIR) recruits and binds to the myeloid differentiation factor 88 (MyD88), which mediates signal transduction to the TNF receptor associated factor 6 (TRAF6) and IL-1 receptor associated kinases (IRAKs). That reaction causes activation of the NF-κB transcription factor, which stimulates gene transcription leading to the production of pro-inflammatory cytokines (Dinarello *et al.,* 2013; Kali-

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**Abbreviations**: BCG, Bacillus Calmette-Guérin; GM-CSF, granulocyte macrophage colony stimulating factor; IL-18, Interleukin 18; IL-18BP, IL-18 binding protein; IL-18R, IL-18 receptor; IL-1RAcP, IL-1 receptor accessory protein; IRAKs, IL-1 receptor-associated kinases; MyD88, myeloid differentiation factor 88; NFκβ, nuclear factor κβ; rBCGmIL-18, recombinant BCG strain producing murine IL-18; TIR, Toll-IL1 receptor domain; TRAF-6, tumor necrosis factor receptor-associated factor-6

na *et al.,* 2000; Wei *et al.,* 2014). The IL-18 signal transduction pathway is illustrated in Fig. 1. IL-18 modulates numerous immune reactions mainly by stimulating the IFN-γ production and its modulatory effects depend on the co-existence of IL-18 with IL-12 or IL-15 in the microenvironment (Robinson *et al.,* 2012) These cytokines can increase the expression of the IL-18Rβ receptor, which is crucial for IL-18 signal transduction.

The proinflammatory activity of IL-18 is balanced by a constitutively secreted IL-18 binding protein (IL-18BP) with an extremely high affinity to IL-18, which is significantly higher than that of IL-18Rα. IL-18BP is a member of the Ig superfamily (Novick *et al.,* 2013). By binding IL-18, IL-18BP diminishes the production of IFN-gamma and other proinflammatory cytokines in order to reduce triggering autoimmune responses to infections (Nakanishi *et al.,* 2001). In humans, an increase in disease severity can be associated with an imbalance between IL-18 and IL-18BP, which yields to elevation of the levels of free IL-18 in the circulation (Dinarello *et al.,* 2013). The increase in the levels of IL-18 and/or IL-18BP has been implicated in severity of systemic juvenile idiopathic arthritis, systemic lupus erythematous, myocardial infarction, Crohn's disease, acute kidney injury, inflammatory bowel disease, sepsis and other diseases. The IL-18BP as well as IL-18 neutralizing antibodies, have been used safely in clinical trials in humans. However, it cannot be forgotten that in some models of disease, IL-18 plays a protective role. The broad spectrum of IL-18 functions, as well as the differing levels of the cytokine and IL-18BP that occur in numerous diseases, indicate that both, IL-18 and IL-18BP, can also be useful as the biomarkers in diagnostics (Dinarello *et al.,* 2013).

IL-18 is regarded as a potent regulator of innate and acquired immune responses (Garcie *et al.,* 2003; Dinarello *et al.,* 2013). With the participation of IL-12 or IL-15, IL-18 induces NK activity and directs immunity towards Th1 cell response, characterized by the profound IFN-gamma production. Without IL-12 or IL-15, IL-18 does not induce IFN-gamma production because these two cytokines increase the expression of IL-18Rβ, which is essential for the IL-18 signal transduction (Dinarello *et al.,* 2013). It has been also shown that IL-18 promotes IFN-gamma production in synergy with other Th-1-related cytokines, IL-2 and IL-23 (Okamoto *et al.,* 2002, Nakahira *et al.,* 2002, Okazawa *et al.,* 2004). In the absence of IL-12, IL-18 can induce the Th2 response. In *E. coli*-infected mice, IL-18 promoted both, Th1 and Th2 responses (Kinoshita, Kuranaga *et al.,* 2006). Studies performed in double knockout mice of IL-12p40 and IL-18, have shown that IL-18 plays a role in the induction of Th17 cell responses (Lim *et al.,* 2013). It has been suggested that IL-18 activates and enhances IL-17 production in already polarized Th17 cells, in a TCR-independent manner in synergy with IL-23 (Weaver *et al.,* 2006). The IL-18-driven increase in IFN-gamma production is accompanied by the enhancement in T cell proliferation and production of various cytokines (IFN-γ, TNF-α, GM-CSF, IL-14, IL-5, IL-13) by T helper (CD4+) lymphocytes and in activation of cytotoxic T (CD8+) lymphocytes. Multiple intraperitoneal IL-18 injections, but not just a single injection, enhanced both Th1 and Th2 response, humoral immunity, as well as neutrophil phagocytic activity in immunocompromised mice infected with pathogens, such as *E. coli, Listeria monocytogenes, Staphylococcus aureus, Cryptococcus neoformans* (Kinoshita *et al.,* 2013). However, exogenous IL-18 may sometimes induce exaggerated inflammatory reactions that are harmful to the host, because of its potent IFN-gamma inducing capability. The excessive IL-18-driven reaction sometimes causes multiorgan injures and lethality.

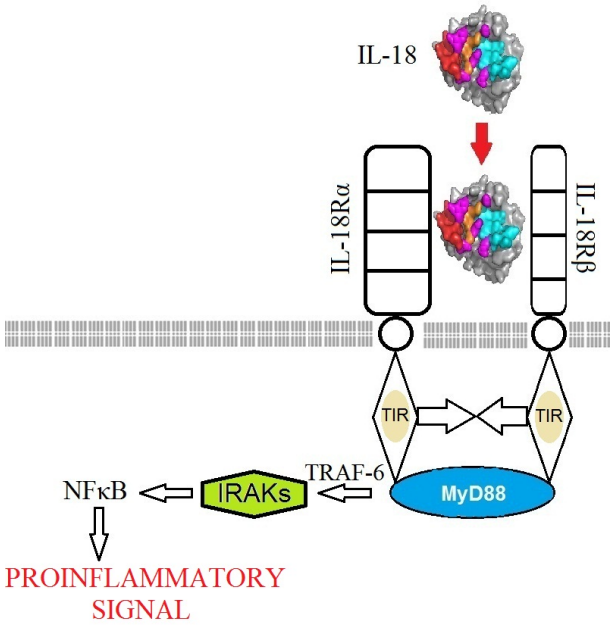


**Figure 1. IL-18 signal transduction pathway (Dinarello *et al.*, 2003, modified).**
IL-18R, IL-18 receptor; TIR, Toll-IL1 receptor domain; MyD88, myeloid differentiation factor 88; TRAF-6, tumor necrosis factor receptor-associated factor-6; IRAKs, IL-1 receptor-associated kinases; NFκB, nuclear factor κB.

## PROSPECTS FOR THE IL-18 APPLICATION IN IMMUNE INTERVENTIONS

The IL-18 driven intensification of IFN-gamma production is accompanied by an increase in nitrogen oxide synthase and killing ability of macrophages. It suggests an important role of IL-18 in the resistance to intracellular pathogens- which are able to develop inside immune cells, including macrophages. *M.tb,* the causative agent of tuberculosis (TB), belongs to this group of bacterial pathogens. Recent epidemiological data clearly indicates that TB remains one of the most deadly infectious diseases. According to the WHO data from 2013, this disease was diagnosed in more than 9 million cases worldwide, and up to 2 million people die annually because of it. The control of TB is still difficult because of not fully effective diagnosis and insufficient protective effectiveness of the only currently used anti-tuberculosis BCG (Bacillus Calmette-Guerin) vaccine (WHO, Global Tuberculosis Report, 2014). A crucial role of IL-18 in the host protection against *M.tb* infection was shown by Kinjo *et al.* in studies using IL-18 knockout and IL-18 transgenic mice. IL-18 deficient mice were more prone to an *M.tb* infection and their sera, spleens, lungs and livers contained less IFN-γ than those of wild-type mice (Kinjo *et al.,* 2002). The IFN-γ production by spleen cells stimulated with mycobacterial antigens was also impaired in IL-18 knockout mice. In contrast, IL-18 transgenic mice were more resistant to an *M.tb* infection than control wild mice, and the levels of IFN-γ in their serum and its production by mycobacterial antigen stimulated spleen cells were increased. These data suggested a significant contribution of IL-18 to the development of Th1 immunity (Kinjo *et al.,* 2002). The pronounced

role of IL-18 in the defense against TB was confirmed by Schneider *et al.* (Schneider *et al.,* 2010). The protective Th1 response to *M.tb* was decreased in IL-18 deficient mice, which constituted a privilege for mycobacterial propagation. Neutrophil driven lung immunopathology, concomitant with unrestricted growth of *M.tb* bacteria, was most probably responsible for the premature death of IL-18 knockout mice infected with *M.tb.* In humans, IL-18 promoter gene –607C/A polymorphism was found to be a risk factor for TB in the Chinese population, but not for the south Indian population (Li *et al.,* 2013, Harishankar *et al.,* 2007). A large case-control study revealed that polymorphisms in the IL-18 receptor alpha chain gene *IL-18R1* were associated with the risk of TB in older Chinese people (over 46 years old) (Zhang *et al.,* 2014). In addition, SNPs (Single Nucleotide Polymorphism) in the *IL-18R1* promoter were associated with the genotype-specific methylation status and genotype-specific *IL-18R1* expression, which suggests that increased DNA methylation and decreased mRNA expression of *IL-18R1* might partially mediate the increased susceptibility to TB.

Extraordinary susceptibility to infections' complications, such as sepsis in patients with severe surgical stress, i.e. trauma injury, burn injury or major surgery, is a frequent and unresolved problem (Kinoshita *et al.,* 2013). The loss of the physical skin barrier, as well as bacterial translocation from the gut, can cause sepsis in such patients. Bacterial infection can lead to lethal multi-organ injuries, as the host defense system is significantly weakened, which promotes microbial growth. Mice studies suggest a possible medical application for IL-18 in the treatment of post-burn *E. coli* infection. Multiple injections of IL-18 to the burn injured mice remarkably increased the IFN-γ production by mononuclear liver cells, thus improving bacterial clearance and mouse survival after *E. coli* infection (Kinoshita *et al.,* 2004; Kinoshita, Kuranaga *et al.,* 2006). Small doses of IL-18 also restored the development of specific antibacterial immune responses, preventing infections with *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus,* the most common bacteria in post-burn infections (Kinoshita *et al.,* 2004; Kinoshita, Shinomiva *et al.,* 2006; Kinoshita *et al.,* 2011). IL-18 driven activation of neutrophils was mostly responsible for improved elimination of these pathogens. Therapy involving administration of IL-18 also results in an up-regulation of IFN-γ production by NK cells and recruitment of neutrophils, monocytes and macrophages into infectious foci. IL-18 treatment for burn-injured mice strengthened the host defense against *P. aeruginosa* infection by the up-regulation of natural IgM production in the liver B1 cells, which were characterized as $CD43^{+}CD5^{-}CD23^{-}B220^{dim}$ cells (Kinoshita, Shinomiva *et al.,* 2006). Such antibodies may opsonize bacteria and facilitate their ingestion by phagocytes before specific antibacterial antibodies are produced. Multiple IL-18 injections activate natural IgM-producing B-1 cells in the liver and restore the humoral immunity against bacterial infections after a burn injury. Such activity of IL-18 may also be helpful in preventing the serious complications in pneumococcal respiratory infections in immunocompromised patients (Kinoshita *et al.,* 2013). Altogether, these data suggest that IL-18 treatment may be recognized as an alternative and useful therapeutic tool against infections caused by intracellular and extracellular pathogens, even in individuals with an immunodeficiency.

In some preclinical models IL-18 has been found to have an antitumor activity (Srivastava *et al.,* 2013, Fabbi *et al.,* 2015). It has been shown that systemic administration of IL-18 enhances the regression of a well-established primary tumor by a mechanism that depends on CD8+ T cells, Fas (CD95)/FasL (Fas ligand) interaction and endogenous IFN-γ, particularly in a combination with other cytokines (Robertson *et al.*, 2008). In a combination with monoclonal antibodies (mAB) recognizing the CD20 antigen on B lymphocytes, IL-18 co-stimulates IFN-γ production and antibody-dependent cellular cytotoxicity (ADCC) of NK cells, which are activated through the surface receptors for Fc fragments of the antibody molecules. In this way, IL-18 augments the activity of mAB against B cell leukemias and lymphomas. In other experimental studies, synergistic effects of IL-18 and *M. bovis* BCG bacteria on the IFN-γ Th1 responses were observed in a mouse model of bladder cancer (Luo *et al.,* 2004). BCG has been applied in the treatment of superficial bladder cancer for years, however, 30–50% of patients did not respond to the BCG therapy. To improve the therapeutic efficacy of BCG, a recombinant BCG strain that functionally secretes murine IL-18 (rBCGmIL-18) was developed. BCG bacteria themselves are strong Th1 inducers. Small amounts of IL-18 released by rBCGmIL-18 augmented Th1 immunity in mice, which led to: a) reduced mycobacterial growth in spleen after infection, b) increased production of IFN-γ, TNF-α (tumor necrosis factor-α) and GM-CSF (granulocyte macrophage colony stimulating factor), and decreased secretion of IL-10, by spleen cells stimulated with BCG, c) augmented macrophage cytotoxicity against bladder cancer MBT-2 cells. It can be expected that this feature of recombinant BCG strains, capable of expressing IL-18, might be useful in immunotherapy and prophylaxis of diseases in which Th1 response is desirable (Dinarello *et al.,* 2013; Luo *et al.,* 2004; Novick *et al.*, 2013). This expectation seems to be confirmed by our recent demonstration of a remarkable advantage of recombinant rBCGhIL-18 producing human IL-18 over nonrecombinant BCG in the stimulation of dendritic cells to preferentially trigger strong IFN-γ secretion by naive CD4(+) T cells in healthy humans vaccinated with BCG (Szpakowski *et al.,* 2015). Previously, the rBCGmIL-18 strain producing murine IL-18 had been found to modify the Th2 type responses in a murine model of the ovalbumin-dependent allergic reaction. Following *in vitro* stimulation with an ovalbumin, lymph node cells from rBCGmIL-18-treated mice produced less IL-5 and more IFN-gamma than those of mice injected with nonrecombinant BCG (Biet *et al.,* 2005). After a challenge with ovalbumin, a strong reduction of bronchoalveolar eosinophilia was observed in rBCGmIL-18-injected mice. This activity of the rBCGhIL-18 strain might be helpful in alleviating the symptoms of allergic reactions. The polarized response of Th2 lymphocytes to an allergen is considered to be the main cause of the pathogenesis of asthma (Kowalski *et al.,* 2015).

Some data point to the crucial role of IL-18 in maintaining the homeostasis. A study group of IL-18 deficient mice indicated a predisposition of mice to obesity and other metabolic disorders. These mice were characterized by a significantly higher weight (by 40%) and an increase in the body fat content (over 100%) compared to wild-type animals. Individuals with a defect in the expression of the surface IL-18Rα receptor also showed a predisposition to obesity, diabetes and other metabolic disorders. These disorders were due to the inefficient functioning of the central nervous system region responsible for the regulation of the appetite, which might affect the depo-

sition of fat in the key blood vessels (Dinarello *et al.,* 2013; Novick *et al*., 2013).

The knowledge of the biology system including IL-18, its receptor IL-18R and inhibitor IL-18BP allows to suggest a possibility of alleviating the symptoms of diseases associated with the IFN-γ overproduction, such as systemic lupus erythematosus, Wagner's disease or Crohn's disease, by blocking the IL-18 activity. It is worth emphasizing that blocking the activity of IL-18 may also find applications in the treatment of multiple sclerosis, where IL-18 promotes the expression of a surface vascular cell adhesion molecule 1, attributed to play an important role in the development of the disease (Dinarello *et al.,* 2013; Novick *et al*., 2013; Wei *et al.,* 2014).

Despite all the potentially positive aspects of administration of exogenous IL-18 in preventing various complications in bacterial infections, immunostimulation of antitumor responses or diminishing allergic disorders, this cytokine can also cause an exaggerated inflammatory response due to its potent IFN-γ inducing capability. It limits a possibility of IL-18 therapy only to immunocompromised hosts, where this cytokine may effectively restore the host immune responses without evoking any exaggerated inflammatory processes. In order to overcome these limitations, new methods of IL-18 administration need to be developed to avoid the potential harmful effects of exogenous IL-18. Recombinant BCG mycobacteria producing IL-18 seem to be a good formula for the administration of IL-18 (Biet *et al.,* 2002, Luo *et al.,* 2004).

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## REFERENCES

Biet F, Kremer L, Wolowczuk I, Delacre M, Locht C (2002) Mycobacterium bovis producing interleukin-18 increases antigen-specific gamma interferon production in mice. *Infect Immun* **70**: 6549–6557.

Biet F, Duez C, Kremer L, Marquilles P, Amniai L, Tonnel AB, Locht C, Pestel J (2005) Recombinant *Mycobacterium bovis* BCG producing IL-18 reduces IL-5 production and bronchoalveolar eosinophilia induced by an allergic reaction. *Allergy* **60**: 1065–1072.

Diakowska D, Markocka-Maczka K, Grabowski K, Lewandowski A (2006) Serum interleukin-12 and interleukin-18 levels in patients with oesophageal squamous cell carcinoma. *Exp Oncol* **28**: 319–322.

Dinarello C, Fantuzzi G (2003) Interleukin-18 and host defense against infection. *J Infect Dis* **187**: S370–S384. doi: 10.1086/374751.

Dinarello CA, Novick D, Kim S, Gilles G (2013) Interleukin-18 and IL-18 binding protein. *Front Immunol* **4**: 289. doi: 10.3389/fimmu.2013.00289.

Fabbi M, Carbotti G, Ferrini S (2015) Context-dependent role of IL-18 in cancer biology and counter-regulation by IL-18BP. *J Leuk Biol* **97**: 665–675. doi: 10.1189/jlb.5RU0714-360RR.

Garcie JA, Robertson SE, Mclnnes IB (2003) Interleukin-18. *J Leuk Biol* **73**: 213–224. doi: 10.1189/jlb.0602313

Harishankar M, Selvaraj P, Rajeswari DN, Anand SP, Narayanan PR (2007) Promoter polymorphism of IL-18 gene in pulmonary tuberculosis in South Indian population. *Int J Immunogenet* **34**: 317–320. doi: 10.1111/j.1744-313X.2007.00714x.

Kalina U, Ballas K, Koyama N, Kauschat D, MMiething C, Arnemann J, Martin H, Hoelzer D, Ottmann OG (2000) Genomic organization and regulation of the human interleukin-18 gene. *Scand J Immunol* **52**: 525–530. doi: 10.1111/j.1365-3083.2000.00836x.

Kinjo Y, Kawakami K, Uezu K, Yara S, Miyagi K, Koguchi Y, Hoshino T, Okamoto M, Kawase Y, Yokota K, Yoshino K, Takeda K, Akira S, Saito A (2002) Contribution of IL-18 to Th1 response and host defense against infection by *Mycobacterium tuberculosis*: a comparative study with IL-12p40. *J Immunol* **169**: 323–329. doi: 10.4049/jimmunol.169.1.323.

Kinoshita M, Seki S, Ono S, Shinomiya N, Hiraide H (2004) Paradoxical effect of IL 18 therapy on the severe and mild *Escherichia coli* infections in burn-injured mice. *Annals Surgery*, **240**: 313–320. doi: 10.1097/01.sla.0000133354.44709.28.

Kinoshita M, Kuranaga N, Matsumoto A, Ono S, Shinomiya N, Hiraide H, Seki S (2006) Multiple interleukin-18 injections promote both mouse Th1 and Th2 responses after sublethal *Escherichia coli* infection. *Clin Exp Immunol* **143**: 41–49. doi: 10.1111/j.1365 2249.2005.02973.x.

Kinoshita M, Shinomiya N, Ono S, Tsujimoto H, Kawabata T, Matsumoto A, Hiraide H, Seki S (2006) Restoration of natural IgM production from liver B cells by exogenous IL-18 improves the survival of burn-injured mice infected with *Pseudomonas aeruginosa*. *J Immunol* **177**: 4627–4635. doi: 10.4049/ jimmunol.177.7.4627.

Kinoshita M, Miyazaki H, Ono S, Inatsu A, Nakashima H, Tsujimoto H, Shinomiya N, Saitoh D, Seki S (2011) Enhancement of neutrophil function by interleukin-18 therapy protects burn-injured mice from methicillin-resistant *Staphylococcus aureus*. *Infect Immun* **79**: 2670–2680. doi: 10.1128/IAI.01298-10.

Kinoshita M, Miyazaki H, Ono S, Seki S (2013) Immunoenhancing therapy with interleukin-18 against bacterial infection in immunocompromised hosts after severe surgical stress. *J Leukoc Biol* **93:** 689–698. doi: 10.1189/jlb.1012502.

Kowalski ML, Makowska JS (2015) Seven steps to the diagnosis of NSAIDs hypersensitivity: how to apply a new classification in real practice? *AAIR* **7**: 312–320. doi: 10.4168/aair.2015.7.4.312.

Lebel-Binay S, Berger A, Zinzindohoué F, Cugnenc P, Thiounn N, Fridman WH, Pagès F (2000) Interleukin-18: biological properties and clinical implications. *Eur Cytokine Netw* **11**: 15–26. doi: 10.4168/aair.2015.7.4.312.

Li DD, Jia LQ, Guo SJ, Shen YC, Wen FQ (2013) Interleukin-18 promoter gene 607C/A polymorphism and tuberculosis risk: a meta-analysis. *Chin Med J* **126**: 3360–3363.

Lim HX, Hong HJ, Jung MY, Cho D, Kim TS (2013) Principal role of IL-12p40 in the decreased Th1 and Th17 responses driven by dendritic cells of mice lacking IL-12 and IL 18. *Cytokine* **63**: 179–186. doi: 10.1016/j.cyto.2013.04.029.

Luo Y, Yamada H, Chen X, Ryan AA, Evanoff DP, Triccas JA, O'Donnell MA (2004) Recombinant *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) expressing mouse IL-18 augments Th1 immunity and macrophage cytotoxicity. *Clin Exp Immunol* **137**: 24–34. doi: 10.1111/j.1365-2249.2004.02522.x.

Nakahira M, Ahn HJ, Park WR, Gao P, Tomura M, Park CS, Hamaoka T, Ohta T, Kurimoto M, Fujiwara H (2002) Synergy of IL-12 and IL-18 for IFN-gamma gene expression: IL-12 induced STAT4 contributes to IFN-gamma promoter activation by up-regulating the binding activity of IL-18-induced activator protein 1. *J Immunol* **168**: 1146–1153. doi: 10.4049/jimmunol.168.3.1146.

Nakamura K, Okamura H, Wada M, Nagata K, Komatsu T, Tamura T (1989) Endotoxin induced serum factor that stimulates gamma interferon production. *Infect Immun* **57**: 590–595.

Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* **12**: 53–72. http://dx.doi.org/10.1016/S1359-6101(00)00015-0.

Novick D, Kim S, Kaplanski G, Dinarello CA (2013) Interleukin-18, more than a Th1 cytokine. *Sem Immunol* **25**: 439–448. doi: 10.1016/j.smim.2013.10.014.

Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* **378**: 88–91. doi:10.1038/378088a0.

Okamoto M, Kato S, Oizumi K, Kinoshita M, Inoue Y, Hoshino K, Akira S, McKenzie AN, Young HA, Hoshino T (2002) Interleukin 18 (IL-18) in synergy with IL-2 induces lethal lung injury in mice: a potential role for cytokines, chemokines, and natural killer cells in the pathogenesis of interstitial pneumonia. *Blood* **99**: 1289–1298. http://dx.doi.org/10.1182/blood.V99.4.1289.

Okazawa **A**, Kanai T, Nakamaru K, Sato T, Inoue N, Ogata H, Iwao Y, Ikeda M, Kawamura T, Makita S, Uraushihara K, Okamoto R, Yamazaki M, Kurimoto M, Ishii H, Watanabe M, Hibi T (2004) Human intestinal epithelial cell-derived interleukin (IL)-18, along with IL-2, IL-7 and IL-15, is a potent synergistic factor for the proliferation of intraepithelial lymphocytes. *Clin Exp Immunol* **136**: 269–276. doi: 10.1111/j.1365-2249.2004.02431.x.

Robertson MJ, Kirkwood JM, Logan TF (2008) A dose-escalation study of recombinant human interleukin-18 using two different schedules of administration in patients with cancer. *Clin Cancer Res* **14**: 3462–3469. doi: 10.1158/1078-0432.CCR-07 4740.

Robinson CM, Jung JY, Nau GJ (2012) Interferon-γ, tumor necrosis factor, and interleukin-18 cooperate to control growth of *Mycobacterium tuberculosis* in human macrophages. *Cytokine* **60**: 233–241. doi:10.1016/j.cyto.2012.06.012.

Schneider BE, Korbel D, Hagens K, Koch M, Raupach B, Enders J, Kaufmann SHE, Mittrucker HW, Schaible UE (2010) A role for IL-18 in protective immunity against *Mycobacterium tuberculosis*. *Eur J Immunol* **40**: 396–405. doi: 10.1002/eji.200939583.

Srivastava S, Pelloso D, Feng H, Voiles L, Lewis D, Haskova Z, Whitacre M, Trulli S, Chen YJ, Toso J, Jonak ZL, Chang HC, Rob-

ertson MJ (2013) Effects of interleukin 18 on natural killer cells: costimulation of activation through Fc receptors for immunoglobulin. *Cancer Immunol Immunother* **62**: 1073–1082. doi: 10.1007/s00262 013-1403-0.

Szpakowski P, Biet F, Locht C, Paszkiewicz M, Rudnicka W, Druszczynska M, Allain F, Fol M, Pestel J, Kowalewicz-Kulbat M (2015) Dendritic cell activity driven by recombinant *Mycobacterium bovis* BCG producing human IL-18, in healthy BCG vaccinated adults. *J Immunol Res* **2015**: 359153. doi: 10.1155/2015/359153.

Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM (2006) Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* **24**: 677–688. doi: http://dx.doi.org/10.1016/j.immuni.2006.06.002.

Wei H, Wang D, Qian Y, Liu X, Fan S, Yin HS, Wang X (2014) Structural basis for the specific recognition of IL-18 by its alpha receptor. *FEBS Letters* **558**: 3838–3843. doi: 10.1016/j.febslet.2014.09.019.

World Health Organization. Global tuberculosis report 2014. www.who.int/tb/publications/global_report/en.

Zhang J, Zheng L, Zhu D, An H, Yang Y, Liang Y, Zhao W, Ding W, Wu X (2014) Polymorphisms in the interleukin 18 receptor 1 gene and tuberculosis susceptibility among Chinese. *PLoS ONE* **9:** e110734. doi:10.1371/journal.pone.0110734.

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*Review Article*

# Inflammasomes in *Mycobacterium tuberculosis*-Driven Immunity

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The development of effective innate and subsequent adaptive host immune responses is highly dependent on the production of proinflammatory cytokines that increase the activity of immune cells. The key role in this process is played by inflammasomes, multimeric protein complexes serving as a platform for caspase-1, an enzyme responsible for proteolytic cleavage of IL-1$\beta$ and IL-18 precursors. Inflammasome activation, which triggers the multifaceted activity of these two proinflammatory cytokines, is a prerequisite for developing an efficient inflammatory response against pathogenic *Mycobacterium tuberculosis* (*M.tb*). This review focuses on the role of NLRP3 and AIM2 inflammasomes in *M.tb*-driven immunity.

## 1. Introduction

*Mycobacterium tuberculosis* (*M.tb*), the causative agent of tuberculosis (TB), is a facultative intracellular bacterium that can survive and replicate within host macrophages [1, 2]. By avoiding critical components of macrophage-killing repertoire such as phagosome-lysosome fusion, phagosome acidification, activity of lysosomal enzymes or reactive oxygen, and nitrogen intermediates, *M.tb* evades killing and eradication [3]. In addition to phagocytic activity and ability to present antigens to T-cells, macrophages are key cells that regulate the antimycobacterial immune response via secreted cytokines. The functional capacity of macrophages in fighting infection depends on the degree of their activation. Inactive macrophages have limited ability to inhibit the growth of ingested mycobacteria, thereby serving as a safe life niche. After activation by interferon-gamma (IFN-$\gamma$) that is secreted by T-cells, macrophages acquire enhanced bactericidal strength enabling them to kill mycobacteria growing intracellularly [4]. The IFN-$\gamma$-driven antimicrobial properties of phagocytes are augmented by IL-18 and IL-1$\beta$, two proinflammatory cytokines processed by caspase-1 that are recruited to the inflammasomes, multiprotein platforms composed inter alia of intracellular sensors for pathogen- or host-derived molecules. IL-18, belonging to the IL-1 family, is produced by a wide range of immune and nonimmune cells [5–7]. The IL-18 precursor (pro-IL-18) is converted by caspase-1 into an active molecule, which forms a signaling complex with IL-18R [8, 9]. The receptor is composed of two chains: alpha (IL-18Ra) and beta (IL-18Rb). IL-18Rb is a signal transduction chain, essential for the formation of a high affinity complex and cell activation. The primary role of IL-18 is to induce IFN-$\gamma$ production in cooperation with IL-12 or IL-15, although immunological effects exerted by IL-18 are dependent on the cytokine microenvironment. IL-18 is able to polarize T lymphocyte response towards Th1, induce T-cell proliferation, activate NK cells, enhance CD8(+) T cytolytic activity, and augment, apart from IFN-$\gamma$, the production of varied cytokines including tumor necrosis factor-α (TNF-α), interleukin- (IL-) 4, IL-5, IL-13, IL-17, and granulocyte-macrophage colony stimulating factor (GM-CSF) [8, 10, 11]. Thus, the multifaceted activity of IL-18 seems to play a prominent role in host defense against both extracellular and intracellular pathogens, including *M.tb*. However, an excessive IL-18 response might contribute to the induction of pathomechanisms leading to the damage of

cells and tissues [12, 13]. Therefore, the proinflammatory activity of IL-18 is balanced by a constitutively secreted IL-18 binding protein (IL-18BP), whose binding to IL-18 decreases the production of IFN-$\gamma$ and other cytokines, thereby reducing the risk of immunopathology [14]. The other inflammasome-dependent cytokine, IL-1$\beta$, which is mainly produced by monocytes and macrophages, plays an important role in inflammation and host immune response by affecting the function of various cells, either alone or in combination with other cytokines [15–17]. The activity of IL-1$\beta$ is tightly regulated at the levels of its transcription and release. The production of IL-1$\beta$ is regulated by several proteins including pyrin, PI-9 (the caspase-1 inhibitor proteinase inhibitor 9), and some CARD-containing proteins, which interfere with the recruitment of caspase-1 or directly neutralize its activity [18]. The effects of IL-1$\beta$ are exerted via binding specific cell surface receptors—IL-1RI and IL-1RII [19]. As in the mature IL-18 form, active IL-1$\beta$ is created after the proteolytic cleavage of its precursor by inflammasome-dependent caspase-1. Mature IL-1$\beta$ plays important homeostatic functions in organisms and is implicated in the initiation of antimicrobial immunity via the induction of TNF-α and IL-6 release and polarization of Th17 response, which improve protective mucosal host defense by the secretion of IL-17 and IL-22 [20, 21]. The proinflammatory role of IL-1$\beta$ in the resistance against *M.tb* has been confirmed by the observation that IL-1$\beta$ or IL-1R knockout mice were found to be more susceptible to TB showing high mortality and increased bacterial burden in the lungs [22]. Additionally, double-deficient IL-1α/$\beta$ mice had significantly larger granulomas, and their alveolar macrophages produced less nitric oxide than the cells from wild-type animals [23].

## 2. Inflammasomes—Mediators of Inflammation

Inflammation is an evolutionarily conserved protective response to noxious stimuli mounted by the innate immune system of the host. Immune deficiencies leading to insufficient development of inflammation processes may result in severe and recurrent infections, although overly intense activation of the inflammation cascade may be a cause of chronic systemic inflammatory disorders [24, 25]. The development of innate immunity starts from the recognition of conservative antigenic structures called DAMPs (danger-associated molecular patterns) and PAMPs (pathogen-associated molecular patterns) by pattern recognition receptors (PRRs) presented on the surface of first-line defense immune cells—macrophages and neutrophils. Activation of these receptors triggers a cascade of signals that results in the induction of multiple proinflammatory cytokines. The final step of the activation is the production of oxygen and nitrogen radicals, essential elements of the intracellular killing system. The secretion of these radicals is under strict control of a variety of monocyte/macrophage-derived cytokines such as IL-1$\beta$ and IL-18. The key role in this process is played by structures called inflammasomes, multimeric protein complexes that control many aspects of innate and adaptive immunity. Through their cooperation with PRRs, inflammasomes activate host defense pathways resulting in clearance of various viral and bacterial infections, including those caused by mycobacteria. They function as an activating scaffold for inflammatory caspases that play an essential role in the maturation and secretion of proinflammatory cytokines as well as in pyroptosis, an inflammatory death of infected cells [26, 27]. Caspases are produced as inactive proenzymes that dimerize and undergo cleavage to form active molecules. Assembly into dimers, facilitated by various adaptor proteins binding to specific regions of their precursor forms—procaspases, is achieved through inflammasome formation [28]. Activated inflammatory caspases, typically caspase-1, lead to the generation of active IL-1$\beta$, IL-18, and IL-33 from their proprotein precursors. The mature cytokines are engaged in the recruitment of immune cells to the sites of infection and enhancement of the host's defensive responses against invading pathogens [26].

The inflammasomes are activated by multiple recognition receptors, which determine their structure and function. The canonical inflammasome sensors are nucleotide-binding domain–like (NLR) proteins and absent in melanoma 2–like (ALR) proteins and PYRIN. All of them have the ability to assemble inflammasomes and activate the inflammatory caspase-1.

The NLR family contains the NLRPs (or NALPs) and the IPAF (ICE-protease-activating factor) subfamilies [29, 30]. Each NLR molecule (NLRP1, NLRP3, NLRP6, NLRP7, NLRP12, or NAIP/NLRC4) recognizes specific ligands that activate the assembly of the inflammasome. NLR proteins consist of the conserved nucleotide-binding and oligomerization domain (NACHT or NOD), an N-terminal caspase recruitment domain (CARD) or pyrin domain (PYD) or baculovirus inhibitor repeat- (BIR-) like domain, and C-terminal leucine rich repeats (LRRs) [26, 31–35]. LRRs are responsible for the recognition of PAMPs, while the NACHT domain activates proinflammatory cytokine pathways via ATP-dependent oligomerization [26, 29]. The NLRP1 inflammasome has a CARD that activates caspase-1 [36, 37], and therefore the recruitment of ASC is not required to interact directly with procaspase-1. However, it has been shown that the participation of ASC in the process enhanced the activation of the enzyme. In contrast, NLRP3 contains no typical CARD domain that contributes to the activation of caspase-1 through the interaction of the PYD domain of NLRP3 with ASC [25]. Compared with NLRP1 and NLRP3, the IPAF protein does not contain a PYD but instead has a CARD that interacts directly with procaspase-1 without the need for ASC [38].

The members of the ALR group (known as the PYHIN family) are characterized by the presence of the pyrin domain (PYD) and one or two hematopoietic IFN-inducible nuclear antigens with 200 amino acid repeat (HIN-200) domains [26]. The PYD recruits proteins for the formation of inflammasomes, while the HIN domain recognizes and binds to DNA that can be found in the cytosol [26]. The best-known ALRs, absent in melanoma 2 (AIM2) and IFN-$\gamma$

inducible protein 16 (IFI16), function as intracellular immune sensors that detect microbial DNA. The PYHIN proteins differ in their localization in the cell compartments; AIM2 can be found in the cytosol, whereas IFI16 is usually localized in the nucleus [39].

PYRIN, another canonical inflammasome-activating protein, is composed of an N-terminal PYD followed by two central B-box zinc finger and coiled-coil domains and in humans, a C-terminal B30.2/rfp/SPRY domain [40]. PYRIN associates through a PYD-PYD interaction with ASC protein, leading to its oligomerization that results in caspase-1 activation and interleukin-1$\beta$ processing [40]. The activation of the PYRIN inflammasome is induced by the inactivation of RhoA GTPase by bacterial toxins [26, 41]. The process of activation has been detected in both mice and humans, suggesting that the B30.2/rfp/SPRY domain is not necessary for its initiation.

## 3. Inflammasomes in *Mycobacterium tuberculosis* Infection

The inflammasomes have been found to play important roles in host immunity against mycobacteria since it has been found that mice deficient in IL-18, IL-1$\beta$, or IL-1 receptor type I (IL-1R1) are more susceptible to *M.tb* infection [42–46]. Two inflammasomes, containing NLRP3 and AIM2 molecules as sensor proteins, were found to play a crucial role in *M.tb*-induced immunity (Figure 1) [20, 47, 48].

The NLRP3-containing inflammasome can be activated by a wide group of stimuli including whole mycobacterial cells, as well as viruses, fungi, environmental chemical irritants, and host-derived molecules such as extracellular ATP, fibrillar amyloid-$\beta$ peptide, and hyaluronan [22, 49–53]. The NLRP3 inflammasome-activated responses result in the release of significant amounts of caspase-1, which leads to maturation and secretion of IL-1$\beta$ and IL-18 and activation of pyroptosis [26]. The process of NLRP3 activation is triggered by at least two signals: (1) a priming signal eliciting the expression of NLRP3, pro-IL-1$\beta$, and pro-IL-18 genes after TLR stimulation and (2) an activation signal leading to the autocatalytic activation of procaspase-1 and proteolytic cleavage of pro-IL-1$\beta$ and pro-IL-18. In most cell types, NLRP3 priming is a prerequisite for deubiquitination and assembly of the NLRP3 inflammasome. Relocalization of NLRP3 to the mitochondria is followed by the secretion of mitochondrial factors into the cytosol, potassium efflux through membrane ion channels, and release of cathepsin resulting in destabilization of lysosomal membranes. Apoptosis-associated speck-like protein (ASC) plays an important role in the formation of an effective inflammasome. ASC recruits procaspase-1 through its C-terminal caspase recruitment domain (CARD) and interacts with NLRP3 via its pyrin domain (PYD), serving as a bridge between these two molecules. The autocatalysis of procaspase-1 results in its cleavage and transformation into active caspase-1, which in turn cleaves the precursors of two proinflammatory cytokines, IL-1$\beta$ and IL-18, leading to their secretion into the cytoplasm or induction [24, 25, 48, 54, 55]. However, the mechanism of triggering the NLRP3 inflammasome complex activation cascade is still a subject of debate, and at least three models for the process have been proposed. The first suggestion is that the activation mechanism is associated with an efflux of potassium ions out of the cell and a reduction in their intracellular concentration. Such a model of activation occurs in monocytes/macrophages after stimulation with numerous stimuli including ATP, nigericin, bacterial cells, or their components [56, 57]. Recently, NEK7 protein, a member of the family of NIMA-related kinases (NEK proteins), has been identified as an NLRP3-binding protein that acts downstream of potassium efflux to regulate NLRP3 assembly and activation [58]. He et al. demonstrated that in the absence of NEK7, caspase-1 activation and IL-1$\beta$ release were abrogated in response to signals that activate NLRP3 [58]. According to the second suggested mechanism, inflammasome activation is a result of lysosomal membrane damage and release of the phagosome content into cytosol [22, 59]. The third and most accepted model assumes that the induction of the NLRP3 inflammasome complex is caused by mitochondrial reactive oxygen species (ROS) [60–63]. The common final step in all of these models is the release of cathepsins into the cytosol leading to the lysosomal destabilization and conversion of procaspase-1 into a biologically active caspase-1 form. It should also be mentioned that formation of the NLRP3 inflammasome and cytokine release occur independently of transcriptional upregulation [64]. Juliana et al. showed that TLR4 signaling through MyD88 nontranscriptionally primed the NLRP3 inflammasome by its deubiquitination. The mechanism was dependent on mitochondria-derived reactive oxygen species and was involved in the secretion of cytokines, such as IL-18, and other inflammatory mediators such as high-mobility group protein 1 (HMGB1) [64, 65].

The AIM2 (absent in melanoma 2) receptor, possessing a C-terminal HIN-200 domain and an N-terminal pyrin domain (PYD), triggers AIM2 inflammasome activation, inflammatory cell death (pyroptosis), and release of IL-1$\beta$ and IL-18 in response to cytosolic double-stranded (ds) DNA [66, 67]. Studies of gene-targeted AIM2-deficient mice have shown that AIM2 inflammasomes play a role in host defense against viruses and intracellular bacterial pathogens such as listeriae and mycobacteria [68–70]. AIM2 inflammasomes can be activated by DNA sequences having at least 80 base pairs in length in a sequence-independent manner [71, 72]. The HIN-200 and PYD domains take part in forming a complex, which is maintained in an inactive state during homeostasis [71, 73]. Binding of dsDNA to HIN-200 facilitates oligomerization of AIM2, and the resulting conformational change exposes the N-terminal PYD to allow the recruitment of the adaptor protein ASC. The CARD of ASC binds the CARD of procaspase-1, that forms an active AIM2 platform. Upon autoactivation, caspase-1 directs maturation and secretion of proinflammatory cytokines [48, 55, 66, 68, 74].

The latest data suggest that NLRP3- or ASC-deficient animals are characterized by impaired inflammasome formation and increased susceptibility to TB [20, 54, 68, 75, 76]. However, NLRP3$^{-/-}$ and ASC$^{-/-}$ mice produced IL-18 and IL-1$\beta$ levels comparable to those of wild-type mice, which

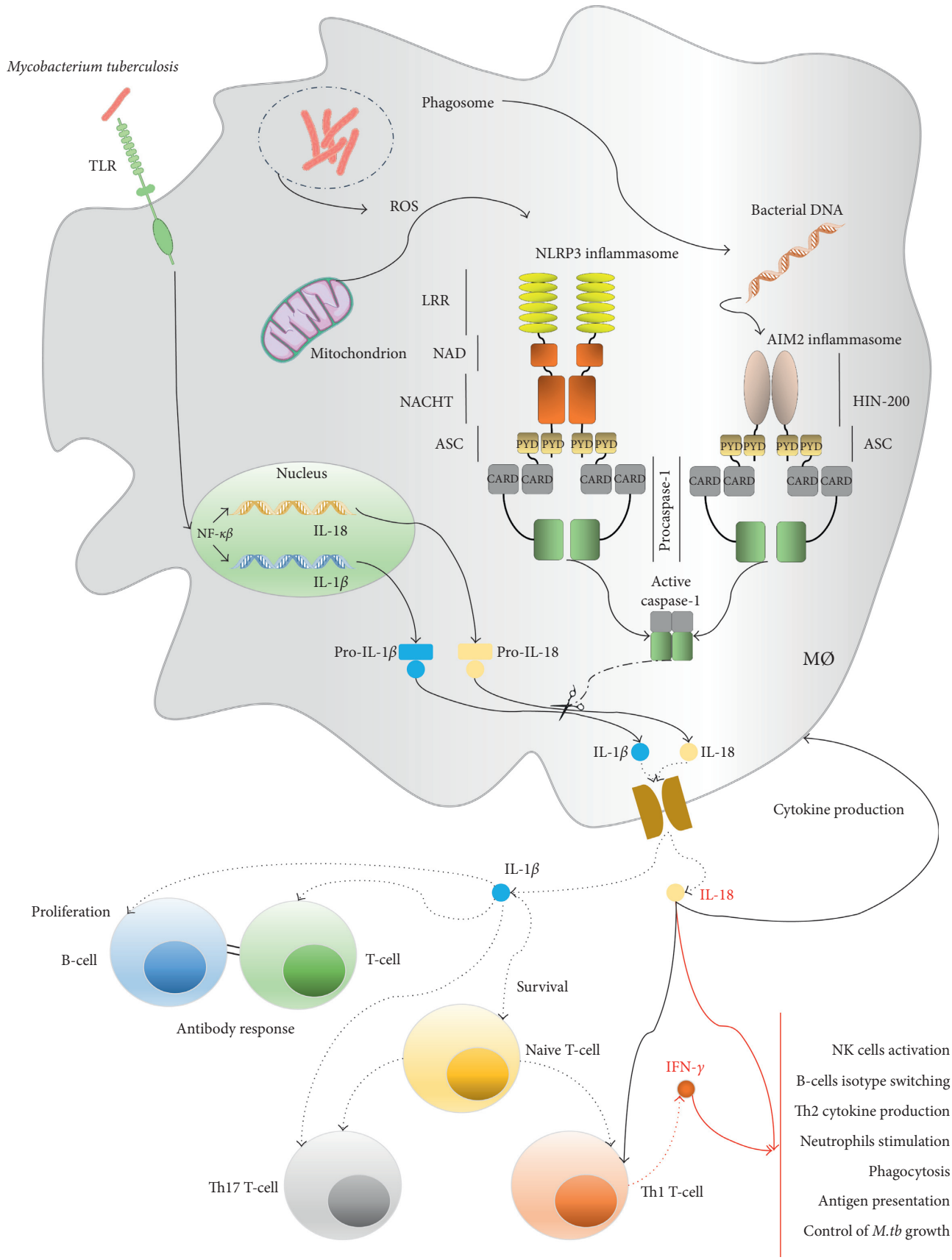


Figure 1: AIM2 and NLRP3 inflammasome activation pathways induced by *Mycobacterium tuberculosis*.

suggests the involvement of inflammasome-independent pathways in the secretion of these cytokines [21, 42, 47]. Many reports have demonstrated that a wide range of microorganisms are able to inhibit inflammasome activation and function. Viruses and many bacterial pathogens develop several mechanisms of repression of inflammasome folding; however, not all mechanisms are clearly understood. *Yersinia enterocolitica* produce YopE and YopT proteins that supress caspase-1 maturation, whereas YopK protein of *Y. pseudotuberculosis* binds to the type III secretion system, thereby preventing the recognition of the pathogen by host cell inflammasome. *Pseudomonas aeruginosa* mediates suppression of NLRC4-inflammasome by secreting ExoU and ExoS effectors, whose mechanism of action still needs elucidation. Virulent *M.tb* can inhibit the formation of AIM2 and NLRP3 inflammasomes both directly and indirectly, but the factors responsible for the inhibition have not been recognized thus far. One of the likely mechanisms is the activity of Zn-metalloprotease called ZMP1, which inhibits the activation of NLRP3 inflammasome and, as a consequence, leads to the reduction of caspase-1 activity [77–79]. Master et al. showed that infection of mice macrophages with zmp1-deleted *M.tb* induces activation of the inflammasome, resulting in enhanced maturation of phagosomes, increased IL-1$\beta$ secretion, and better *M.tb* clearance in lungs [79]. It is probable that *M.tb* is able to restrain the activation of other inflammasome types, but evidence is needed to confirm this hypothesis. In addition to the induction of inflammasome activation via PRRs, *M.tb* antigens can modulate other innate immunity-associated functions. One recently identified protein, tyrosine phosphatase (Ptp) A, enters the nucleus of the host cells and regulates the transcription of many host genes involved in the mechanisms of innate immunity, cell proliferation, and migration [80]. The enzyme is also able to dephosphorylate certain host proteins (p-JNK, p-p38, and p-VPS33B), leading to inhibition of phagosome-lysosome fusion and blocking the acidification of phagosomes. Both activities are crucial for *M.tb* virulence in vivo through the promotion of *M.tb*'s intracellular survival in macrophages [80]. *M.tb* often escapes from the phagosome within a few days of the invasion of the host organism and creates difficulties in assessing the potential role of inflammasomes during the initial stages of mycobacterial infection. Moreover, the evaluation of IL-1$\beta$ and IL-18 produced as a result of inflammasome activation is inadequate in revealing the significance of formed multiprotein platforms in the course of developing infection. The initiation of phagocytosis causes a decrease in the levels of potassium ions in macrophages, which have been found to be one of the crucial inflammasome activators during infections with *M.tb* and nontuberculous mycobacteria [81]. Other regulators such as thioredoxin-interacting proteins, activated by the increase in reactive oxygen species in cytosol, are thought to have minor effect on the formation of inflammasomes in *M.tb* infection [47]. The signaling cascade can also be activated by the mycobacterial type VII secretion system (ESX-1), which is responsible for translocation of extracellular DNA (eDNA) in cytosol and the production of IFN-$\beta$. Many studies have demonstrated that, at the molecular level, IFN-$\beta$ regulates the AIM2 inflammasome activity [82, 83]. Some ESX-1-deficient *M. smegmatis* mutants have been shown to possess limited capacity for AIM2 inflammasome activation. However, in contrast to nontuberculous mycobacteria (NTM), *M.tb* mutants lacking ESX-1 system failed to inhibit AIM2 formation, while the wild-type strain inhibited the inflammasome activation [47, 84]. The suggested mechanism of inhibition involves the IFN-$\beta$-mediated induction of IL-10, which in turn suppresses IL-1$\beta$ production [85, 86]. However, further investigation is needed to elucidate the molecular mechanism of *M.tb*-driven AIM2 inhibition and its consequences for bacterial virulence. *M. bovis* BCG vaccine strain, which does not possess the ESX-1 system, poorly activates multiple NLR and inflammasome complex components including caspase-1 [87]. The bacilli repress the expression of thioredoxin-interacting protein (TXNIP), an antioxidant inhibitor recruiting caspase-1 to the NLRP3 inflammasome. The inhibition of TXNIP by BCG limits NLRP3 activation and restrains pyroptosis following mycobacterial infection. Proinflammatory responses to BCG bacilli was found to be driven primarily through Toll-like receptors (TLRs), since BCG does not activate expression of genes downstream of TLR/MyD88- and NOD-2-driven NF-$\kappa\beta$ and AP-1 pathways. However, BCG is still able to induce moderate IL-1$\beta$ secretion as measured by transcription of inflammasome network genes [87, 88]. Understanding BCG-induced pathways of inflammasome activation can be helpful in improving the existing vaccine or developing new antituberculous vaccines. The recombinant BCG ΔureC::hly vaccine candidate (VPM1002) has been shown to induce improved protection against TB over the parental BCG strain [4]. Saiga et al. demonstrated that VPM1002 activated the AIM2 inflammasome and caspase-1 through the ability of listeriolysin to perforate phagosome membranes, which is encoded by the *hly* gene integrated into BCG genome [4]. The perforation facilitates the release of mycobacterial DNA into the cytosol, in a way that is similar to the ESX-1 system of *M.tb*. Mice vaccinated with VPM1002 showed increased production of IL-1$\beta$ and IL-18 as well as induction of the stimulator of IFN genes (STING)-dependent autophagy, which promotes delivery of BCG antigens to MHC molecules and improves their presentation to T-cells [4].

Apart from direct induction of proinflammatory cytokine secretion, the activated caspase-1 triggers the pyroptotic death of infected cells. The cytosolic protein Gasdermin D (GSDMS) is a key mediator of this process. The cleavage of GSDMD by activated caspase-1 results in the release of its N-terminal fragment (GSDMD-NT), which forms pores in the plasma membrane of the infected cell leading to the elimination of the pathogen [26, 89–91]. The pores disrupt cell membrane integrity allowing water influx, cell swelling, and osmotic lysis together with an efflux of small molecules, including proinflammatory cytokines. GSDMD-NT is able to kill both cell-free and intracellular microorganisms and can be thought as a new antibacterial agent. However, it is still not known whether GSDMD-NT is able to permeabilize the membrane of the phagosomes and kill the bacteria

hidden within these organelles. So far, there is no evidence of such a function. It is probable that the inhibition of bacterial growth is mediated by other caspase components. Using single-cell analysis, Thurston et al. demonstrated that the replication of cytosolic *Salmonella typhimurium* was inhibited independently or prior to the onset of cell death, suggesting that caspase-1 and caspase-11 might have additional functions in the elimination of cytosolic bacteria [92].

## 4. Therapeutics Targeting Inflammasome Pathways

Biologic agents interfering with inflammasome activation may provide new means of therapeutical interventions for many diseases. These agents may target either upstream processes of inflammasome regulation or downstream IL-1 signaling [41]. Inappropriate activity of inflammasomes has been found to be involved in the pathogenesis of certain autoinflammatory skin disorders such as cryopyrin-associated periodic syndrome (CAPS) or familial Mediterranean fever (FMF) as well as a number of chronic inflammatory diseases such as multiple sclerosis, gouty arthritis (gout), atherosclerosis, type 2 diabetes, and obesity [29, 93, 94]. Moreover, mechanisms controlling the NLRP3 inflammasome arrangement have also been implicated in the development of lung, kidney, and liver diseases [95–97]. Colchicine, a drug used for treatment of gout, has been shown to inhibit macrophage NLRP3 inflammasome assembly and activation in vitro and in vivo [98]. Colchicine blocks monosodium urate crystal-induced NLRP3 inflammasome-driven caspase-1 activation and IL-1$\beta$ processing and release, suppresses the expression of genes involved in cell regulation, and inhibits IL-1-induced L-selectin expression on neutrophils [99]. Other therapeutics that target inflammasome-driven end products include VX-765 (inhibitor of caspase-1 activation), Anakinra (recombinant form of IL-1 receptor antagonist), Canakinumab (monoclonal antibody against IL-1$\beta$), Rilonacept (IL-1 inhibitor), IL-18 binding protein, and anti-IL-18 receptors antibodies [8, 41, 100]. A number of new molecules have been identified as inhibitors of IL-1$\beta$ processing (glyburide, parthenolide, CRID3, auranofin, isoliquiritigenin, $\beta$-hydroxybutyrate, and MCC950); however, confirming their clinical utility will require additional time and research [24].

## 5. Conclusion

Inflammasomes have been implicated as specialized signaling platforms critical for the regulation of both innate immunity and inflammation. *M.tb* has been shown to modulate the host innate immune response by delaying cell death systems of the host, thereby facilitating its own proliferation. Understanding the molecular mechanisms of inflammasome activation during intracellular pathogen infections such as with *M.tb*, and the evasive mechanisms employed by this evading pathogen, may lead to development of more potent therapies to combat the proliferation of *M.tb*.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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## References

[1] A. Welin, D. Eklund, O. Stendahl, and M. Lerm, "Human macrophages infected with a high burden of ESAT-6-expressing *M. tuberculosis* undergo caspase-1- and cathepsin B-independent necrosis," *PLoS One*, vol. 6, no. 5, p. e20302, 2011.

[2] T. R. Lerner, S. Borel, and M. G. Gutierrez, "The innate immune response in human *tuberculosis*," *Cellular Microbiology*, vol. 17, no. 9, pp. 1277–1285, 2015.

[3] V. C. Korb, A. A. Chuturgoon, and D. Moodley, "*Mycobacterium tuberculosis*: manipulator of protective immunity," *International Journal of Molecular Sciences*, vol. 17, no. 3, p. 131, 2016.

[4] H. Saiga, N. Nieuwenhuizen, M. Gengenbacher et al., "The recombinant BCG Δ*ureC::hly* vaccine targets the AIM2 inflammasome to induce autophagy and inflammation," *Journal of Infectious Diseases*, vol. 211, no. 11, pp. 1831–1841, 2015.

[5] H. E. Barksby, S. R. Lea, P. M. Preshaw, and J. J. Taylor, "The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders," *Clinical & Experimental Immunology*, vol. 149, no. 2, pp. 217–225, 2007.

[6] D. Novick, S. Kim, G. Kaplanski, and C. A. Dinarello, "Interleukin-18, more than a Th1 cytokine," *Seminars in Immunology*, vol. 25, no. 6, pp. 439–448, 2013.

[7] C. A. Dinarello and F. Giamila, "Interleukin-18 and host defense against infection," *Journal of Infectious Diseases*, vol. 187, no. 2, pp. 370–384, 2003.

[8] C. A. Dinarello, D. Novick, S. Kim, and G. Kaplanski, "Interleukin-18 and IL-18 binding protein," *Frontiers in Immunology*, vol. 4, pp. 1–10, 2013.

[9] C. A. Dinarello, "Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist," *International Reviews of Immunology*, vol. 16, no. 5-6, pp. 457–499, 1998.

[10] R. Gutzmer, K. Langer, S. Mommert, M. Wittmann, A. Kapp, and T. Werfel, "Human dendritic cells express the IL-18R and are chemoattracted to IL-18," *Journal of Immunology*, vol. 171, no. 12, pp. 6363–6371, 2003.

[11] S. Gardella, C. Andrei, S. Costigliolo, A. Poggi, M. R. Zocchi, and A. Rubartelli, "Interleukin-18 synthesis and secretion by dendritic cells are modulated by interaction with antigen-specific T cells," *Journal of Leukocyte Biology*, vol. 66, no. 2, pp. 237–241, 1999.

[12] D. V. Pechkovsky, T. Goldmann, E. Vollmer, J. Müller-Quernheim, and G. Zissel, "Interleukin-18 expression by alveolar epithelial cells type II in tuberculosis and sarcoidosis," *FEMS Immunology & Medical Microbiology*, vol. 46, no. 1, pp. 30–38, 2006.

[13] S. El-Masry, M. Lotfy, W. Nasif, I. El-Kady, and M. Al-Badrawy, "Elevated serum level of interleukin (IL)-18, interferon (IFN)-$\gamma$ and soluble fas in patients with pulmonary complications in tuberculosis," *Acta Microbiologica et Immunologica Hungarica*, vol. 54, no. 1, pp. 65–67, 2007.

[14] K. Nakanishi, T. Yoshimoto, H. Tsutsui, and H. Okamura, "Interleukin-18 regulates both Th1 and Th2 responses," *Annual Review of Immunology*, vol. 19, no. 1, pp. 423–474, 2001.

[15] N. A. Turner, "Effects of interleukin-1 on cardiac fibroblast function: relevance to post-myocardial infarction remodeling," *Vascular Pharmacology*, vol. 60, no. 1, pp. 1–7, 2014.

[16] C. Duque, L. Arroyo, H. Ortega et al., "Different responses of human mononuclear phagocyte populations to *Mycobacterium tuberculosis*," *Tuberculosis*, vol. 94, no. 2, pp. 111–122, 2014.

[17] G. Lopez-Castejon and D. Brough, "Understanding the mechanism of IL-1β secretion," *Cytokine & Growth Factor Reviews*, vol. 22, no. 4, pp. 189–195, 2011.

[18] L. D. Church, G. P. Cook, and M. F. McDermott, "Primer: inflammasomes and interleukin 1β in inflammatory disorders," *Nature Clinical Practice Rheumatology*, vol. 4, no. 1, pp. 34–42, 2008.

[19] M. Ricote, I. García-Tuñón, F. R. Bethencourt, B. Fraile, R. Paniagua, and M. Royuela, "Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1RII, and IL-1Ra) in prostate carcinoma," *Cancer*, vol. 100, no. 7, pp. 1388–1396, 2004.

[20] F. L. van de Veerdonk, M. G. Netea, C. A. Dinarello, and L. A. B. Joosten, "Inflammasome activation and IL-1β and IL-18 processing during infection," *Trends in Immunology*, vol. 32, no. 3, pp. 110–116, 2011.

[21] M. G. Netea, A. Simon, F. van de Veerdonk, B.-J. Kullberg, J. W. M. Van der Meer, and L. A. B. Joosten, "IL-1β processing in host defense: beyond the inflammasomes," *PLoS One*, vol. 6, no. 2, p. e10000661, 2010.

[22] A. Halle, V. Hornung, G. C. Petzold et al., "The NALP3 inflammasome is involved in the innate immune response to amyloid-beta," *Nature Immunology*, vol. 9, no. 8, pp. 857–865, 2008.

[23] H. Yamada, S. Mizumo, R. Horai, Y. Iwakura, and I. Sugawara, "Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice," *Laboratory Investigation*, vol. 80, no. 5, pp. 759–767, 2000.

[24] H. Guo, J. B. Callaway, and J. P. Ting, "Inflammasomes: mechanism of action, role in disease, and therapeutics," *Nature Medicine*, vol. 21, no. 7, pp. 677–687, 2015.

[25] G. dos Santos, M. A. Kutuzov, and K. M. Ridge, "The inflammasome in lung diseases," *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 303, no. 8, pp. 627–633, 2012.

[26] X. Liu and J. Lieberman, "A mechanistic understanding of pyroptosis: the fiery death triggered by invasive infection," *Advances in Immunology*, vol. 135, pp. 81–117, 2017.

[27] D. R. McIlwain, T. Berger, and T. W. Mak, "Caspase functions in cell death and disease," *Cold Spring Harbor Perspectives in Biology*, vol. 5, no. 4, p. a008656, 2013.

[28] F. Martinon, K. Burns, and J. Tschopp, "The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta," *Molecular Cell*, vol. 10, no. 2, pp. 417–426, 2002.

[29] A. J. S. Choi and S. W. Ryter, "Inflammasomes: molecular regulation and implications for metabolic and cognitive diseases," *Molecules and Cells*, vol. 37, no. 6, pp. 441–448, 2014.

[30] B. K. Davis, H. Wen, and J. P. Ting, "The inflammasome NLRs in immunity, inflammation, and associated diseases," *Annual Review of Immunology*, vol. 29, no. 1, pp. 707–735, 2011.

[31] J. P. Ting, R. C. Lovering, E. S. Alnemri et al., "The NLR gene family: a standard nomenclature," *Immunity*, vol. 28, no. 3, pp. 285–287, 2008.

[32] Y. K. Kim, J. S. Shin, and M. H. Nahm, "NOD-like receptors in infection, immunity, and diseases," *Yonsei Medical Journal*, vol. 57, no. 1, pp. 5–14, 2016.

[33] J. Bertin, W. J. Nir, C. M. Fischer et al., "Human CARD4 protein is a novel CED-4/Apaf-1 cell death family member that activates NF-kB," *Journal of Biological Chemistry*, vol. 274, no. 19, pp. 12955–12958, 1999.

[34] N. Roy, M. S. Mahadevan, M. McLean et al., "The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy," *Cell*, vol. 80, no. 1, pp. 167–178, 1995.

[35] K. Schroder and J. Tschopp, "The inflammasomes," *Cell*, vol. 140, no. 6, pp. 821–832, 2010.

[36] B. Faustin, L. Lartigue, J. M. Bruey et al., "Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation," *Molecular Cell*, vol. 25, no. 5, pp. 713–724, 2007.

[37] J. Chavarría-Smith and R. E. F. Vance, "The NLRP1 inflammasomes," *Immunology Reviews*, vol. 265, no. 1, pp. 33-34, 2015.

[38] J. L. Poyet, S. M. Srinivasula, M. Tnani, M. Razmara, T. Fernandes-Alnemri, and E. S. Alnemri, "Identification of Ipaf, a human caspase-1-activating protein related to Apaf-1," *Journal of Biological Chemistry*, vol. 276, no. 30, pp. 28309–28313, 2001.

[39] J. A. Cridland, E. Z. Curley, M. N. Wykes et al., "The mammalian PYHIN gene family: phylogeny, evolution and expression," *BMC Evolutionary Biology*, vol. 12, no. 1, pp. 140–156, 2012.

[40] J.-W. Yu, J. Wu, Z. Zhang et al., "Cryopyrin and pyrin activate caspase-1, but not NF-κβ, via ASC oligomerization," *Cell Death and Differentiation*, vol. 13, no. 2, pp. 236–249, 2006.

[41] C. de Torre-Minguela, P. M. de Castillo, and P. Pelegrin, "The NLRP3 and pyrin inflammasomes: implications in the pathophysiology of autoinflammatory diseases," *Frontiers in Immunology*, vol. 8, p. 43, 2017.

[42] K. D. Mayer-Barber, D. L. Barber, K. Shenderov et al., "Cutting edge: caspase-1 independent IL-1β production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling *in vivo*," *Journal of Immunology*, vol. 184, no. 7, pp. 3326–3330, 2010.

[43] I. Sugawara, H. Yamada, H. Kaneko, S. Mizuno, K. Takeda, and S. Akira, "Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice," *Infection and Immunity*, vol. 67, no. 5, pp. 2585–2589, 1999.

[44] I. Sugawara, H. Yamada, S. Hua, and S. Mizuno, "Role of interleukin (IL)-1 type 1 receptor in mycobacterial infection," *Microbiology and Immunology*, vol. 45, no. 11, pp. 743–750, 2001.

[45] C. M. Fremond, D. Togbe, E. Doz et al., "IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to *Mycobacterium tuberculosis* infection," *Journal of Immunology*, vol. 179, no. 2, pp. 1178–1189, 2007.

[46] B. E. Schneider, D. Korbel, K. Hagens et al., "A role for IL-18 in protective immunity against *Mycobacterium tuberculosis*," *European Journal of Immunology*, vol. 40, no. 2, pp. 396–405, 2010.

[47] V. Briken, S. E. Ahlbrand, and S. Shah, "*Mycobacterium tuberculosis* and the host cell inflammasome: a complex relationship," *Frontiers in Cellular and Infection Microbiology*, vol. 9, no. 3, p. 62, 2013.

[48] R. Wassermann, M. F. Gulen, C. Sala et al., "*Mycobacterium tuberculosis* differentially activates cGAS- and inflammasome-dependent intracellular immune responses through ESX-1," *Cell Host Microbe*, vol. 17, no. 6, pp. 799–810, 2015.

[49] O. Gross, H. Poeck, M. Bscheider et al., "Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence," *Nature*, vol. 459, no. 7245, pp. 433–436, 2009.

[50] J. A. Duncan, X. Gao, M. T. Huang et al., "*Neisseria gonorrhoeae* activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome," *Journal of Immunology*, vol. 182, no. 10, pp. 6460–6469, 2009.

[51] S. Mariathasan, D. S. Weiss, K. Newton et al., "Cryopyrin activates the inflammasome in response to toxins and ATP," *Nature*, vol. 440, no. 7081, pp. 228–232, 2006.

[52] K. Yamasaki, J. Muto, K. R. Taylor et al., "NLRP3/cryopyrin is necessary for interleukin-1beta (IL-1beta) release in response to hyaluronan, an endogenous trigger of inflammation in response to injury," *Journal of Biological Chemistry*, vol. 284, no. 19, pp. 12762–12771, 2009.

[53] H. Watanabe, O. Gaide, V. Pétrilli et al., "Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity," *Journal of Investigative Dermatology*, vol. 127, no. 8, pp. 1956–1963, 2007.

[54] D. Eklund, A. Welin, H. Andersson et al., "Human gene variants linked to enhanced NLRP3 activity limit intramacrophage growth of *Mycobacterium tuberculosis*," *Journal of Infectious Diseases*, vol. 209, no. 5, pp. 749–753, 2014.

[55] P. J. Shaw, M. F. McDermott, and T. D. Kanneganti, "Inflammasomes and autoimmunity," *Trends in Molecular Medicine*, vol. 17, no. 2, pp. 57–64, 2011.

[56] V. Pétrilli, S. Papin, C. Dostert, A. Mayor, F. Martinon, and J. Tschopp, "Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration," *Cell Death and Differentiation*, vol. 14, no. 9, pp. 1583–1589, 2007.

[57] R. Muñoz-Planillo, L. Franchi, L. S. Miller, and G. Núñez, "A critical role for hemolysins and bacterial lipoproteins in *Staphylococcus aureus*-induced activation of the Nlrp3 inflammasome," *Journal of Immunology*, vol. 183, no. 6, pp. 3942–3948, 2009.

[58] Y. He, M. Y. Zeng, D. Yang, B. Motro, and G. Núñez, "NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux," *Nature*, vol. 530, no. 7590, pp. 354–369, 2016.

[59] V. Hornung, F. Bauernfeind, and A. Halle, "Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization," *Nature Immunology*, vol. 9, no. 8, pp. 847–856, 2008.

[60] M. E. Heid, P. A. Keyel, C. Kamga et al., "Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation," *Journal of Immunology*, vol. 191, no. 10, pp. 5230–5238, 2013.

[61] M. T. Sorbara and S. E. Girardin, "Mitochondrial ROS fuel the inflammasome," *Cell Research*, vol. 21, no. 4, pp. 558–560, 2011.

[62] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, "A role for mitochondria in NLRP3 inflammasome activation," *Nature*, vol. 469, no. 7329, pp. 221–225, 2011.

[63] K. Nakahira, J. A. Haspel, V. A. Rathinam et al., "Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome," *Nature Immunology*, vol. 12, no. 3, pp. 222–230, 2011.

[64] H. M. Lee, J. J. Kim, H. J. Kim et al., "Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes," *Diabetes*, vol. 62, no. 1, pp. 194–204, 2013.

[65] C. Juliana, T. Fernandes-Alnemri, S. Kang, A. Farias, F. Qin, and E. S. Alnemri, "Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation," *Journal of Biological Chemistry*, vol. 287, no. 43, pp. 36617–36622, 2012.

[66] V. Hornung, A. Ablasser, M. Charrel-Dennis et al., "AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC," *Nature*, vol. 458, no. 7237, pp. 514–518, 2009.

[67] T. Fernandes-Alnemri, J. W. Yu, P. Datta, J. Wu, and E. S. Alnemri, "AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA," *Nature*, vol. 458, no. 7237, pp. 509–513, 2009.

[68] H. Saiga, S. Kitada, Y. Shimada et al., "Critical role of AIM2 in *Mycobacterium tuberculosis* infection," *International Immunology*, vol. 24, no. 10, pp. 637–644, 2012.

[69] V. A. K. Rathinam, Z. Jiang, S. N. Waggoner et al., "The AIM2 inflammasome is essential for host-defense against cytosolic bacteria and DNA viruses," *Nature Immunology*, vol. 11, no. 5, pp. 395–402, 2010.

[70] J.-D. Sauer, C. E. Witte, J. Zemansky, B. Hanson, P. Lauer, and D. A. Portnoy, "*Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol," *Cell Host & Microbe*, vol. 7, no. 5, pp. 412–419, 2010.

[71] T. L. Roberts, A. Idris, J. A. Dunn et al., "HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA," *Science*, vol. 323, no. 5917, pp. 1057–1060, 2009.

[72] T. Jin, A. Perry, J. Jiang et al., "Structures of the HIN domain: DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor," *Immunity*, vol. 36, no. 4, pp. 561–571, 2012.

[73] T. Jin, A Perry, P. Smith, J. Jiang, and T. S. Xiao, "Structure of the absent in melanoma 2 (AIM2) pyrin domain provides insights into the mechanisms of AIM2 autoinhibition and inflammasome assembly," *Journal of Biological Chemistry*, vol. 288, no. 19, pp. 13225–13235, 2013.

[74] S. M. Man, R. Kark, and T. D. Kanneganti, "AIM2 inflammasome in infection, cancer, and autoimmunity: role in DNA sensing, inflammation, and innate immunity," *European Journal of Immunology*, vol. 46, no. 2, pp. 269–280, 2016.

[75] R. P. Lai, G. Meintjes, K. A. Wilkinson et al., "HIV-tuberculosis-associated immune reconstitution inflammatory syndrome is characterized by Toll-like receptor and inflammasome signaling," *Nature Communications*, vol. 6, p. 8451, 2015.

[76] W. C. Chao, C. L. Yen, and Y. H. Wu, "Increased resistin may suppress reactive oxygen species production and inflammasome activation in type 2 diabetic patients with pulmonary tuberculosis infection," *Microbes and Infection*, vol. 17, no. 3, pp. 195–204, 2015.

[77] M. Galle, P. Schotte, M. Haegman et al., "The *Pseudomonas aeruginosa* type III secretion system plays a dual role in the regulation of caspase-1 mediated IL-1beta maturation," *Journal of Cellular and Molecular Medicine*, vol. 12, no. 5, pp. 1767–1776, 2008.

[78] I. E. Brodsky, N. W. Palm, S. Sadanand et al., "A *Yersinia* effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system," *Cell Host & Microbe*, vol. 7, no. 5, pp. 376–387, 2010.

[79] S. S. Master, S. K. Rampini, A. S. Davis et al., "*Mycobacterium tuberculosis* prevents inflammasome activation," *Cell Host & Microbe*, vol. 3, no. 4, pp. 224–232, 2008.

[80] J. Wang, P. Ge, L. Qiang et al., "The mycobacterial phosphatase PtpA regulates the expression of host genes and promotes cell proliferation," *Nature Communications*, vol. 8, no. 1, p. 244, 2017.

[81] T. D. Kanneganti, M. Lamkanfi, and Y. G. Kim, "Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signalling," *Immunity*, vol. 26, no. 4, pp. 433–443, 2007.

[82] J. W. Jones, N. Kayagaki, P. Broz et al., "Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 21, pp. 9771–9776, 2010.

[83] K. Tsuchiya, H. Hara, I. Kawamura et al., "Involvement of absent in melanoma 2 in inflammasome activation in macrophages infected with *Listeria monocytogenes*," *Journal of Immunology*, vol. 185, no. 2, pp. 1186–1195, 2010.

[84] S. Shah, A. Bohsali, S. E. Ahlbrand et al., "Cutting edge: *Mycobacterium tuberculosis* but not nonvirulent mycobacteria inhibits IFN-β and AIM2 inflammasome-dependent IL-1β production via its ESX-1 secretion system," *Journal of Immunology*, vol. 191, no. 7, pp. 3514–3518, 2013.

[85] K. D. Mayer-Barber, B. B. Andrade, D. L. Barber et al., "Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection," *Immunity*, vol. 35, no. 6, pp. 1023–1034, 2011.

[86] A. Novikov, M. Cardone, R. Thompson et al., "*Mycobacterium tuberculosis* triggers host type I IFN signaling to regulate IL-1β production in human macrophages," *Journal of Immunology*, vol. 187, no. 5, pp. 2540–2537, 2011.

[87] H. B. Huante, S. Gupta, V. C. Calderon et al., "Differential inflammasome activation signatures following intracellular infection of human macrophages with *Mycobacterium bovis* BCG or *Trypanosoma cruzi*," *Tuberculosis*, vol. 110, pp. S35–S44, 2016.

[88] A. Dorhoi, G. Nouailles, S. Jörg et al., "Activation of the NLRP3 inflammasome by *Mycobacterium tuberculosis* is uncoupled from susceptibility to active tuberculosis," *European Journal of Immunology*, vol. 42, no. 2, pp. 374–384, 2012.

[89] T. Bergsbaken, S. L. Fink, and B. T. Cookson, "Pyroptosis: host cell death and inflammation," *Nature Reviews Microbiology*, vol. 7, no. 2, pp. 99–109, 2009.

[90] L. Sborgi, S. Rühl, E. Mulvihill et al., "GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death," *EMBO Journal*, vol. 35, no. 16, pp. 1766–1778, 2016.

[91] M. M. Gaidt and V. Hornung, "Pore formation by GSDMD is the effector mechanism of pyroptosis," *EMBO Journal*, vol. 35, no. 20, pp. 2167–2169, 2016.

[92] T. L. M. Thurston, S. A. Matthews, E. Jennings et al., "Growth inhibition of cytosolic *Salmonella* by caspase-1 and caspase-11 precedes host death," *Nature Communications*, vol. 7, p. 13292, 2016.

[93] J. Lukens, V. D. Dixit, and T.-D. Kanneganti, "Inflammasome activation in obesity-related inflammatory diseases and autoimmunity," *Discovery Medicine*, vol. 12, no. 62, pp. 65–74, 2014.

[94] P. Gurung and T.-D. Kanneganti, "Autoinflammatory skin disorders: the inflammasome in focus," *Trends in Molecular Medicine*, vol. 22, no. 7, pp. 545–564, 2016.

[95] D. De Nardo, C. M. De Nardo, and E. Latz, "New insights into mechanisms controlling the NLRP3 inflammasome and its role in lung disease," *American Journal of Pathology*, vol. 184, no. 1, pp. 42–54, 2014.

[96] G. Szabo and T. Csak, "Inflammasomes in liver diseases," *Journal of Hepatology*, vol. 57, no. 3, pp. 642–654, 2012.

[97] H. J. Anders and D. A. Muruve, "The inflammasomes in kidney disease," *Journal of the American Society of Nephrology*, vol. 22, no. 6, pp. 1007–1018, 2011.

[98] A. P. Demidowich, A. I. Davis, N. Dedhia, and J. A. Yanovski, "Colchicine to decrease NLRP3-activated inflammation and improve obesity-related metabolic dysregulation," *Medical Hypotheses*, vol. 92, pp. 67–73, 2016.

[99] G. Nuki, "Colchicine: its mechanism of action and efficacy in crystal-induced inflammation," *Current Rheumatology Reports*, vol. 10, no. 3, pp. 218–227, 2008.

[100] A. A. Jesus and R. Goldbach-Mansky, "IL-1 blockade in autoinflammatory syndromes," *Annual Review of Medicine*, vol. 65, no. 1, pp. 223–244, 2014.

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Regular paper

# Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population

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**Background: Polymorphisms in genes encoding cytokines are known to determine susceptibility to *Mycobacterium tuberculosis* (*M.tb*) infection. In particular, interleukin-18 (IL-18), an inducer of interferon-gamma (IFN-γ), playing an important role in anti-mycobacterial immune responses, may influence the risk of developing active tuberculosis (TB). Aim: A case-control study was performed to investigate whether two promoter polymorphisms of the IL-18 gene at positions -137G/A (rs187238) and -607A/C (rs1946518) affect the serum level of IL-18 and might be associated with genetic susceptibility to tuberculosis (TB) in the Polish population. Methods: Two IL-18 gene promoter SNPs were detected by an allele-specific polymerase chain reaction. Serum IL-18 levels were measured immunoenzymatically using Human Total IL-18 ELISA DuoSet (R&D). Results: A single-gene analysis showed no differences either in allele or genotype frequencies of the studied SNPs between TB patients and healthy controls. No significant differences in the frequencies of any of the haplotypes between TB patients and healthy controls were found. None of the polymorphic variants of IL-18(-137G/A) or IL-18(-607A/C) SNP was associated with IL-18 producing capability. Conclusion: Our results suggest that IL-18(-137G/A) and IL-18(-607A/C) polymorphisms may not be risk factors for susceptibility to TB in the Polish population. Increased serum IL-18 level observed in TB patients has no genetic background, but is a consequence of *M.tb* infection. Further studies with a higher sample size are required to confirm these findings.**

**Key words**: interleukin-18 (IL-18), tuberculosis, gene polymorphism

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**Abbreviations**: BCG vaccine, Bacillus Calmette-Guérin vaccine; *M.tb*, *Mycobacterium tuberculosis*; SNPs, single nucleotides polymorphisms; TB, tuberculosis

## INTRODUCTION

Interactions between cytokines and their influence on the activity of the human immune system remain an important subject of many scientific studies (Etna *et al.,* 2014; Sobti *et al.,* 2011). Among the interlinking network of interactions between cytokines, one of the important nodes is interleukin 18, belonging to the family of interleukin 1 and expressed by a range of inflammatory cell types. The protein, described for the first time in 1995 and named "IFN-gamma inducing factor", is a pleiotropic cytokine produced by various hematopoietic and non-hematopoietic cells. IL-18 takes part in a number of reactions in the human body and has countless roles ranging from its involvement in maintaining the homeostasis, through not always positive involvement in the development of autoimmune diseases, to a significant role in the prevention of infectious diseases such as tuberculosis (TB) and many others (Akdis *et al.,* 2016; Dinarello *et al.,* 2013; Wawrocki *et al.,* 2016). Among numerous infectious diseases, pulmonary tuberculosis has been a serious health concern for many years, despite the large-scale prophylactic vaccination with the BCG vaccine (Bacillus Calmette-Guérin) introduced in 1921. A significant problem regarding the incidence of TB is co-infection with the HIV virus and a successive increase in the frequency of isolation of multi-drug resistant TB (MDR-TB). According to the latest data from the World Health Organization, approximately 1.7 billion people in the world are infected with *Mycobacterium tuberculosis* (*M.tb*). These infections usually take a latent form, however, in at least 10% of cases they can transform into the active form of the disease. This problem is growing due to the increasing number of people with immunodeficiencies such as patients with autoimmune diseases and cancers, who take immunosuppressive drugs, as well as elderly people who, because of their age, are at a higher risk of reactivation of the latent *M.tb* infection. The interactions between mycobacteria and the human body at the molecular and cellular levels are still poorly understood. Recent studies demonstrated the existence of polymorphisms within the regulatory sequences of the interleukin 18 gene, which is located at chromosome 11q22.2-22.3 position and consists of five introns and six exons. More specifically, several differences in single nucleotides can alter the way transcription factors are joined, thereby influencing the expression and the immunological activity of IL-18. SNP-type mutations in the -607C/A (rs1946518) and -137G/C (rs187238) positions of the IL-18 promoter region seem to be particularly interesting due to the fact that a change from cytosine to adenine at position -607 may negatively affect the transduction factor cAMP and activation of adenyl cyclase, while nucleotide change at position -137 involving the conversion of guanine into cytosine may influence the activity of the nuclear factor H4TF-1 and result in a potential decrease in gene expression (Giedraitis *et al.,* 2001; Pawlik *et al.,* 2009). As was shown, IL-18 deficiency increases the susceptibility to mycobacterial infection. Kinjo and others (Kinjo

*et al.,* 2002) demonstrated that IL-18 knockout mice, characterized by attenuated production of IFN-γ, were more prone to *M.tb* infection than wild-type individuals. Therefore, genetic variations within the IL-18 gene might potentially predispose to the development of TB in humans through their influence on the expression of the cytokine and, subsequently, IFN-γ-mediated Th1 response.

Single nucleotides polymorphisms (SNPs) in many cytokine-encoding genes are attributed to ethnicity and occur frequently in the Far East populations. Literature data on the occurrence of such SNPs among Caucasians is contradictory (Akdis *et al.,* 2016; Giedraitis *et al.,* 2001; Zhou *et al.,* 2015). In our study, we tested the hypothesis whether two promoter polymorphisms of the IL-18 gene at positions -137G/A and -607A/C might contribute to TB susceptibility in the Polish population. We compared the allele and genotype frequencies of polymorphic variants between TB patients and healthy controls as well as assessed their serum concentration of IL-18.

## MATERIALS AND METHODS

**Study subjects**. A study group consisted of 192 HIV-negative adults vaccinated with *M. bovis* BCG including 68 patients with pulmonary tuberculosis (37 males, 31 females) aged 21–81 years hospitalized in the Regional Center Hospital for Tuberculosis, Lung Diseases and Rehabilitation in Lodz, Poland and 124 healthy volunteers (38 males, 86 females) aged 18–84 years, who had never had tuberculosis. TB diagnosis was established by Ziehl-Neelsen staining of sputum smears and *M.tb* culture as well as chest radiography and standard clinical examination by infectious disease consultants. All subjects had given written informed consent to participate in the study, which was approved by the ethics committee. Blood samples obtained from the volunteers were used for DNA isolation and assessment of polymorphisms in the promoter region of the IL-18 gene, whereas serum was used to assess the level of IL-18 protein.

**DNA isolation**. Genetic material was isolated from 2 ml of peripheral blood obtained from all volunteers using EDTA tubes and the BD Vacutainer® Blood Collection system. DNA isolation was based on the use of a commercial QIAamp® DNA Blood Mini set and was carried out from the buffy coat obtained after centrifugation (150×*g*, 4°C, 10 min). The isolation process was fully compliant with the manufacturer's guidelines enclosed in the kit's instructions and carried out within no more than 2 hours from the collection of the blood sample. The extracted DNA was stored at –80°C until analyzed.

**Quantification of serum IL-18 level**. Serum in a volume of approximately 1 ml was obtained from subjects using BD Vacutainer® Blood Collection system tubes and was used for evaluation of IL-18 protein level by Human Total IL-18 DuoSet ELISA (R&D) according to the manufacturer's instructions.

**IL-18 SNPs genotyping**. Polymerase chain reactions (PCRs) were performed using the primers for 2 promoter SNPs: rs187238 (-137G/A) and rs1946518 (-607A/C). For -137G/A SNP: 5'-CCCCAACGGAAGCTTTTAAAAAG-3' (-137G allele-specific), 5'-CCCCAACGGAAGCTTTTAAAAAC-3' (-137A allele-specific) and 5'-AGGAGGTGCGCAAAAACTGG-3' (common reverse) primers were used to amplify a 261-bp product. A control primer 5'-CCAATACTGGGAATTATTCCGCA-3 ' and the same common reverse primer were used to yield a 446-bp product as an internal positive amplification control (Fig. 1A). For -607A/C SNP: 5'-GTTGAAGCAAGTGTAATTATTAAAAA-3' (-607A allele-specific), 5'-GCAGAAGTTAGTGTAAAAATTATTAC-3' (-607C allele-specific) and 5'-CCTTAACATCTTGGATCACC-3 ' (common reverse) primers were used to amplify a 196-bp product. A control primer 5'-CTTTGCCATTATTCCAGGAA-3' and the same common reverse primer were used to yield a 301-bp product as an internal positive amplification control (Fig. 1B) (Giedraitis, 2001). Reactions were performed in a Biometra PCR thermocycler using Bio-Rad reagents including iProof™ High-Fidelity DNA Polymerase. At the first step, denaturation was performed for 2 min at 94°C, followed by seven cycles of 94°C for 20s, 64°C for 40s and 72°C for 40s and after that 25 cycles of 94°C for 20s, 57°C for 40s, 72°C for 40 s. The PCR products were visualized by electrophoresis in a 2% agarose gel stained with ethidium bromide using a documentation system Gel Doc 2000 (Bio-Rad).

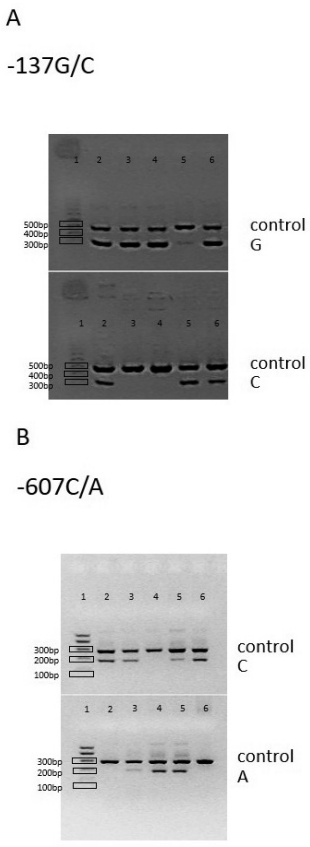


**Figure 1. PCR products of -137G/A (A) and -607A/C (B) SNPs visualized under UV light after staining with ethidium bromide.** The electrophoresis was done on 2% agarose gel at 5 volt/cm for 2 hours. Lane 1: DNA molecular weight marker, lanes 2–6: samples.

**Statistical analysis**. Statistical analyses were done using Statistica 13.0 PL software (StatSoft, Poland). Hardy-Weinberg equilibrium (HWE) was tested using a web-based program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Differences in allelic and genotypic frequencies between TB patients and healthy volunteers were compared by Pearson's chi-square test or two-tailed Fisher's exact tests when analyzing frequencies were lower than five to determine statistically significant differences between the studied groups. Odds ratios (OR) with the respective 95% confidence intervals (95% CI)

**Table 1. Distribution of alleles and genotypes of the -137G/C polymorphism in the IL-18 gene**

| -137G/C | | TB n=68 | HC n=124 | OR (95% CI) | *p* |
|---|---|---|---|---|---|
| Allele | G | 93 (68%) | 172 (69%) | 0.96 (0.61–1.50) | 0.84 |
| | C | 43 (32%) | 76 (31%) | | |
| Genotype | G/G | 32 (47%) | 60 (48%) | 1 | 0.86 |
| | G/C | 29 (43%) | 52 (42%) | 1.05 (0.56–1.95) | 0.92 |
| | C/C | 7 (10%) | 12 (10%) | 1.09 (0.39–3.05) | 0.89 |
| | $p^{HWE}$ | 1.00 | 0.84 | | |

**Table 2. Distribution of alleles and genotypes of the -607C/A polymorphism in the IL-18 gene**

| -607C/A | | TB n=68 | HC n=124 | OR (95% CI) | *p* |
|---|---|---|---|---|---|
| Allele | C | 89 (65%) | 151 (61%) | 1.22 (0.78–1.88) | 0.37 |
| | A | 47 (35%) | 97 (39%) | | |
| Genotype | C/C | 27 (40%) | 42 (34%) | 1 | 0.42 |
| | C/A | 35 (51%) | 67 (54%) | 0.81 (0.43–1.53) | 0.73 |
| | A/A | 6 (9%) | 15 (12%) | 0.62 (0.21–1.80) | 0.48 |
| | $p^{HWE}$ | 0.42 | 0.19 | | |

were also calculated. Probability values of 0.05 or less were regarded as statistically significant. Differences in serum IL-18 levels were analyzed using the Mann-Whitney U-test. The logistic regression analysis done for the important main and interaction effects was performed using the SNPstats software (http://bioinfo.iconcologia.net/SNPstats).

## RESULTS

### Genotype and allele distribution of polymorphisms in the *IL-18* promoter region among TB patients and healthy controls

The distribution of IL-18-(137G/A)or IL-18(-607A/C) genotypes in TB and HC groups is shown in Tables 1 and 2. There was no evidence to reject the Hardy-Weinberg equilibrium (HWE) hypothesis in both TB and HC groups at the standard significance level of 0.05.

The dominant homozygous G/G IL-18(-137G/C) genotype was the most frequent among the studied groups occurring in 47% (32/68) TB patients and 48% (60/124) healthy volunteers (Table 2). The frequency of G/C IL-18(-137G/C) genotype in the groups under study was also similar, at 43% (29/43) among TB and 42% (52/124) among HC subjects. The C/CIL-18(-137G/C) genotype was equally uncommon (10%) in TB and HC individuals (Table 1).

Similarly to IL-18(-137G/C) genotypes, IL-18(-607C/A) gene variants were comparatively distributed among TB and HC groups (Table 2). The heterozygous C/A IL-18(-607C/A) genotype was found in 51% (35/67) patients with TB and 54% (67/124) healthy controls, whereas C/C IL-18(-607C/A) homozygosity occurred in 40% (27/68) TB patients and 34% (42/124) healthy individuals. The least frequent genotype was the A/A IL-18(-607C/A) variant (Table 2).

### Haplotype frequencies analysis

A pair-wise nucleotide analysis revealed no significant differences in the frequencies of four haplotypes between TB patients and healthy controls (Table 3). The "CG" haplotype was the most common in both studied groups, occurring in 60.6% of TB patients and 57.6% of healthy controls. The rarest "CC" haplotype was found in 4.8% of TB patients and 3.2% of healthy individuals (Table 3).

### Effect of IL-18 gene polymorphism on serum IL-18 level

Statistical analysis of the serum IL-18 levels in the studied groups showed a significantly higher concentration of IL-18 among TB patients (880.2±1079.1 pg/ml) compared to healthy controls (467.4±462.4 pg/ml)

**Table 3. Results of the IL-18 haplotype analysis in the studied population.**

| Haplotype | IL-18 SNP | | Haplotype statistics | | | | |
|---|---|---|---|---|---|---|---|
| | -607C/A | -137G/C | Frequency (%) | | | OR (95% CI) | *p*-value |
| | | | Total | TB patients | Healthy controls | | |
| 1 | C | G | 58.7 | 60.6 | 57.6 | 1.00 | – |
| 2 | A | C | 27.2 | 26.8 | 27.4 | 0.89 (0.54-1.47) | 0.65 |
| 3 | A | G | 10.3 | 7.7 | 11.6 | 0.58 (0.25-1.34) | 0.20 |
| 4 | C | C | 3.8 | 4.8 | 3.2 | 1.44 (0.45-4.58) | 0.54 |

**Table 4. Effect of IL-18 gene promoter -137G/C and -607C/A polymorphisms on serum IL-18 level.**

| SNP | | IL-18 [pg/ml] | | *p* |
|---|---|---|---|---|
| | | TB n=68 | HC n=124 | |
| IL-18 -137G/C | | | | |
| Genotype | G/G | 835.3±1321.3 | 434.7±318.4 | $p=0.02$ |
| | G/C | 874.3±618.2 | 477.9±572.8 | $p<0.001$ |
| | C/C | 1100.3±1458.0 | 584.9±551.9 | $p=0.43$ |
| IL-18-607C/A | | | | |
| Genotype | C/C | 901.1±1429.2 | 434.2±296.0 | $p=0.03$ |
| | C/A | 822.5±762.5 | 415.0±329.1 | $p<0.001$ |
| | A/A | 1123.0±964.1 | 793.9±990.5 | $p=0.30$ |

($p<0001$). Our results showed higher IL-18 levels in the sera from homozygous -137C/C and -607A/A carriers from both groups when compared to the concentrations in the sera from the other individuals, however, the observed differences were not statistically significant ($p>0.05$) (Table 4).

## DISCUSSION

Most of the scientific reports in the past focused mainly on pathogen-host interactions, attributing an increase in susceptibility to the misfortune and unique features of the pathogen. Genetic factors and predispositions were closely related only to such diseases as cancer and cardiovascular diseases, and no one linked them to infectious diseases. Today, we know that these assumptions were not correct, studies from the late '80s clearly showed that the genetic background can be even more important in the development of infectious diseases than in the cardiovascular disabilities (Möller & Hoal, 2010).

The best way to determine human susceptibility to infectious diseases is a comprehensive analysis of the genome through a large-scale sequencing of the genetic material from many cases and controls. This method has many advantages and allows tracing any potential mutations and genetic changes affecting the incidence of disease. However, this approach has also many drawbacks such as high cost and potential difficulties in analyzing a huge amount of data representative for all the world's populations (Möller & Hoal, 2010; Hirschhorn & Daly, 2005).

After finding the evidence for linking the selected genome region to the susceptibility to the disease, genetic and physical maps are created based on the case studied to narrow the chromosome interval and allow gene identification or positional cloning in complex diseases where the function of the involved gene is not exactly known nor well described. In the case of pulmonary TB, recent linkage studies described many chromosomal regions which contain some genetic variations that affect the susceptibility of specific populations to the disease. This is worth emphasizing because the research units were most often able to find the characteristic variations only for a given group. Valuable scientific research and literature reports concerning susceptibility to pulmonary TB include: study on the population of Gambia and South Africa indicating the chromosome regions 15q and Xq described by Bellamy and others in 2000 (Bellamy *et al.,* 2000), analysis of patients from Morocco and the 8q12-q13 chromosomal region described by Baghdadi and others in 2006 (Baghdadi *et al.,* 2006) or the Thai population described by Mahasirimongkol in 2009 (Mahasirimongkol, 2009) with the 5q23.2-q31.3 region and many others. These experiments were also based on the evaluation of many other parameters and diagnostic tests. What is worth pointing out is the fact that all the mentioned research papers described different regions of the human genome. The observed lack of similarity in the occurrence of susceptibility regions in these studies is probably due to the combination of many factors. Moreover, the sought-after genetic markers of susceptibility to TB are unique to a specific family or population, making them impossible to be confirmed in the studies on other populations. This confirms the thesis that this type of approach is possible only through a large financial outlay and examination of a very large group consisting of TB patients and healthy controls from numerous populations. Therefore, it seems more practical to focus on one selected gene whose expression corresponds, for example, to the production of a selected cytokine or which is known for its involvement in the development of the studied disease such as TB. This approach allows the assessment of susceptibility and focusing only on the key elements associated with the development of disease and immunity. A significant role is played by the occurrence of SNP-type mutations in genes of cytokines directly related to the development of TB. IFN-γ, encoded by the *IFNG* gene, is secreted by Th1 cells as the main cytokine, which activates macrophages and is critical for controlling the development of *M.tb* infection. Epidemiological studies, including extensive meta-analysis, suggest links between *IFNG* polymorphism at +874 T/A (rs2430561) position and TB development. *IFNG* polymorphism is the most common studied polymorphism in terms of TB susceptibility, sites, and severity, however, those associations are still the subject of research due to population differences (Baghdadi *et al.,* 2006; Bellamy *et al.,* 2000; Hashemi *et al.,* 2011; Mahasirimongkol *et al.,* 2006; Shen *et al.,* 2017; Wei *et al.,* 2017). Other cytokine polymorphisms determining the outcome of *M.tb* infection are located in genes encoding TNF-α (-308G/A, -863C/A, -857C/T and -238G/A), IL-12 (641A/G, 684C/T, 1094T/C, and 1132G/C) and IL-10 (-1082G/A, -819T/C, and -592A/C) (Fol *et al.,* 2015; Han *et al.,* 2011; Kim *et al.,* 2012; Liang *et al.,* 2014; Yi *et al.,* 2015). In our study, we assessed the frequency of two polymorphic variants (-137G/C and -607C/A) in the promoter of gene encoding IL-18, the most powerful inducer of IFN-γ. The polymorphism at position -137 was described by Giedraitis and others in 2001 (Giedraitis *et al.,*

2001), who showed that the occurrence of dominant homozygous genotypes resulted in an increase in TB incidence up to 1.6 times. Our observations did not confirm these results. Although the dominant homozygous IL-18-137G/G genotype was the most frequent in the whole population, there was no difference in the frequency of the genotype occurrence among TB patients (47%) and healthy controls (48%). Interestingly, the percentage of the IL-18-137G allele in the Polish population (68.5%) was found to be lower than that in the Chinese Han population (86%). The diversity of the allele distribution may partially explain the differences in TB susceptibility between races. Two other polymorphic variants – IL-18-137G/C and IL-18-137C/C were distributed similarly among the studied groups of Poles. All these data suggest that IL-18(-137G/C) SNP does not contribute to TB susceptibility in the Polish population. Similarly, the results by Zhou and others (Zhou *et al.,* 2015) did not show any significant differences in the distribution of the C/C(-137G/C) genotype, however, the frequency of the G/G(-137G/C) genotype among the Chinese was significantly higher in the TB group than in the healthy control group. The authors suggested that the IL-18-137G allele might be a predisposing gene of TB, while the IL-18-137C allele played a role in preventing *M.tb* infection. The explanation for these discrepancies is difficult to find. Identified cytokine polymorphisms might be population-specific and associated with the resistance to mycobacterial infection in some but not all world's populations suggesting that they may serve as markers of TB susceptibility only in specific ethnic groups (Delgado *et al.,* 2002; Zhou *et al.,* 2015).

In our study, neither genotypes nor allele frequencies of IL-18(-607C/A) SNP showed differences between TB patients and healthy controls. These observations are consistent with the results obtained by Harishankar and others, Taheri and others and Zhou and others in the South India, Southeast Iran, and Chinese Han populations (Harishankar *et al.,* 2007; Taheri *et al.,* 2012; Zhou *et al.,* 2015). The proportion of the IL-18-607C allele in the Polish population (63%) is similar to that observed among the Iranians (58.8%) or the Chinese (53.3%).

To assess the effects of the IL-18 gene promoter SNPs on the expression of IL-18 protein and, consequently, on TB susceptibility, we measured IL-18 levels in the sera from the studied individuals. Our data did not confirm any effect of either IL-18-(137G/C) or IL-18(-607C/A) polymorphisms on IL-18 producing capability. Serum levels of IL-18 from each of the carriers were similar, however, in the group of TB patients, the observed concentrations of the cytokine were significantly higher than those measured for the controls. It indicates that the increase in the serum IL-18 level observed in TB patients has no genetic background, but is probably a consequence of *M.tb* infection. Lack of association between serum IL-18 concentrations and the IL-18 genotype was also observed by Evans and others (Evans *et al.,* 2007). On the contrary, Zhouand others (Zhou *et al.,* 2015) demonstrated that IL-18-137G/C polymorphism influenced the production of IL-18 by peripheral blood mononuclear cells (PBMCs) and showed that PBMCs from IL-18-137C/C individuals produced higher levels of IL-18 than those with the IL-18-137G/G genotype. Several studies also reported that alleles of the IL-18(-607C/A) SNP are associated with different promoter activity of the IL-18 gene (Sivalingam *et al.,* 2003; Takada *et al.,* 2002). An example of such research is the study by Takada and co-workers, who demonstrated that in the Japanese population the C allele was correlated with the greater transcriptional activity of the IL-18 promoter resulting in the higher expression of IL-18 (Takada *et al.,* 2002). However, our results are contradictory to those reporting such a relationship. The discrepancies could be due to many reasons. First of all, a low sample size of our study could have resulted in the limited power of detection of the studied genetic effect. Secondly, it is possible that the studied SNPs do not determine disease susceptibility directly, but are in linkage disequilibrium with other functional but so far unknown alleles. Finally, the differences in SNP-associated susceptibility may be ethnicity-related and present in specific populations all over the world.

In summary, our results suggest that IL-18(-137G/A) and IL-18(-607A/C) polymorphisms may not be the risk factors for susceptibility to TB in the Polish population. It will be utmost interesting to validate these findings with a higher sample size and to perform functional studies assessing the transcriptional activity of the IL-18 gene.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## REFERENCES


Akdis M, Aab A, Altunbulakli C, Azkur K, Costa RA, Crameri R, Duan S, Eiwegger T, Eljaszewicz A, Ferstl R, Frei R, Garbani M, Globinska A, Hess L, Huitema C, Kubo T, Komlosi Z, Konieczna P, Kovacs N, Kucuksezer UC, Meyer N, Morita H, Olzhausen J, O'Mahony L, Pezer M, Prati M, Rebane A, Rhyner C, Rinaldi A, Sokolowska M, Stanic B, Sugita K, Treis A, van de Veen W, Wanke K, Wawrzyniak M, Wawrzyniak P, Wirz OF, Zakzuk JS, Akdis CA (2016) Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* **138**: 984–1010. https://doi.org/10.1016/j.jaci.2016.06.033

Baghdadi J El, Orlova M, Alter A, Ranque B, Chentoufi M, Lazrak F, Archane MI, Casanova J-L, Benslimane A, Schurr E, Abel L (2006) An autosomal dominant major gene confers predisposition to pulmonary tuberculosis in adults. *J. Exp. Med.* **203**: 1679–1684. https://doi.org/10.1084/jem.20060269

Bellamy R, Beyers N, McAdam KPWJ, Ruwende C, Gie R, Samaai P, Bester D, Meyer M, Corrah T, Collin M, Camidge DR, Wilkinson D, Hoal-van Helden E, Whittle HC, Amos W, van Helden P, Hill AVS (2000) Genetic susceptibility to tuberculosis in Africans: A genome-wide scan. *Proc. Natl. Acad. Sci.* **97**: 8005–8009. https://doi.org/10.1073/pnas.140201897

Delgado JC, Baena A, Thim S, Goldfeld AE (2002) Ethnic-specific genetic associations with pulmonary tuberculosis. *J. Infect. Dis.* **186**: 1463–1468. https://doi.org/10.1086/344891

Dinarello CA, Novick D, Kim S, Kaplanski G (2013) Interleukin-18 and IL-18 binding protein. *Front. Immunol.* **4**: 1–10. https://doi.org/10.3389/fimmu.2013.00289

Etna MP, Giacomini E, Severa M, Coccia EM (2014) Pro-and anti-inflammatory cytokines in tuberculosis: A two-edged sword in TB pathogenesis. *Semin. Immunol.* **26**: 543–551. https://doi.org/10.1016/j.smim.2014.09.011

Evans J, Collins M, Jennings C, van der Merwe L, Söderström I, Olsson T, Levitt NS, Lambert E V, Goedecke JH (2007) The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. *Eur. J. Endocrinol.* **157**: 633–640. https://doi.org/10.1530/EJE-07-0463

Fol M, Druszczynska M, Wlodarczyk M, Ograczyk E, Rudnicka W (2015) Immune response gene polymorphisms in tuberculosis. *Acta Biochim. Pol.* **62**: 633–640. https://doi.org/10.18388/abp.2015_1130

Giedraitis V, He B, Huang W-X, Hillert J (2001) Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J. Neuroimmunol.* **112**: 146–152. https://doi.org/10.1016/S0165-5728(00)00407-0

Han M, Yue J, Lian Y, Zhao Y, Wang H, Liu L (2011) Relationship between single nucleotide polymorphism of interleukin-18 and susceptibility to pulmonary tuberculosis in the Chinese Han population. *Microbiol. Immunol.* **55**: 388–393. https://doi.org/10.1111/j.1348-0421.2011.00332.x

Harishankar M, Selvaraj P, Rajeswari DN, Anand SP, Narayanan PR (2007) Promoter polymorphism of IL-18 gene in pulmonary tuber-

culosis in South Indian population. *Int. J. Immunogenet.* **34**: 317–320. https://doi.org/10.1111/j.1744-313X.2007.00714.x

Hashemi M, Sharifi-Mood B, Nezamdoost M, Moazeni-Roodi A, Naderi M, Kouhpayeh H, Taheri M, Ghavami S (2011) Functional polymorphism of interferon-γ (IFN-γ) gene +874T/A polymorphism is associated with pulmonary tuberculosis in Zahedan, Southeast Iran. *Prague Med. Rep.* **112**: 38–43

Hirschhorn JN, Daly MJ (2005) Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.* **6**: 95–108. https://doi.org/10.1038/nrg1521

Kim H-L, Cho SO, Kim S-Y, Kim S-H, Chung W-S, Chung S-H, Kim S-S, Ko S-G, Jeong C-H, Kim S-J, Hong S-H, Um J-Y (2012) Association of interleukin-18 gene polymorphism with body mass index in women. *Reprod. Biol. Endocrinol.* **10**: 31. https://doi.org/10.1186/1477-7827-10-31

Kinjo Y, Kawakami K, Uezu K, Yara S, Miyagi K, Koguchi Y, Hoshino T, Okamoto M, Kawase Y, Yokota K, Yoshino K, Takeda K, Akira S, Saito A (2002) Contribution of IL-18 to Th1 response and host defense against infection by Mycobacterium tuberculosis: a comparative study with IL-12p40. *J. Immunol.* **169**: 323–329

Liang B, Guo Y, Li Y, Kong H (2014) Association between IL-10 gene polymorphisms and susceptibility of tuberculosis: Evidence based on a meta-analysis. *PLoS One* **9**: e88448. https://doi.org/10.1371/journal.pone.0088448

Mahasirimongkol S, Chantratita W, Promso S, Pasomsab E, Jinawath N, Jongjaroenprasert W, Lulitanond V, Krittayapoositpot P, Tongsima S, Sawanpanyalert P, Kamatani N, Nakamura Y, Sura T (2006) Similarity of the allele frequency and linkage disequilibrium pattern of single nucleotide polymorphisms in drug-related gene loci between Thai and northern East Asian populations: implications for tagging SNP selection in Thais. *J. Hum. Genet.* **51**: 896–904. https://doi.org/10.1007/s10038-006-0041-1

Möller M, Hoal EG (2010) Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. *Tuberculosis* **90**: 71–83. https://doi.org/10.1016/j.tube.2010.02.002

Pawlik A, Kurzawski M, Drozdzik M, Dziedziejko V, Safranow K, Herczynska M (2009) Interleukin-18 gene (*IL18*) promoter polymorphisms in patients with rheumatoid arthritis. *Scand. J. Rheumatol.* **38**: 159–165. https://doi.org/10.1080/03009740802600748

Shen L, Liang Z, Xu C, Yang J, Han X, Zhao H, Liu A, Bao F (2017) Association between interleukin-18-137G/C gene polymorphisms and tuberculosis risk in chinese population: A meta-analysis. *J. Clin. Cell. Immunol.* **08**: 1–5. https://doi.org/10.4172/2155-9899.1000514

Sivalingam SP, Yoon KH, Koh DR, Fong KY (2003) Single-nucleotide polymorphisms of the interleukin-18 gene promoter region in rheumatoid arthritis patients: protective effect of AA genotype. *Tissue Antigens* **62**: 498–504.

Sobti R, Sharma V, Abitew A, Berhane N, Mahdi S, Askari M, Kuttiat V, Wanchu A (2011) IL-18 Gene promoter region 607C/A polymorphism in HIV-1 infected north indian population. *Balk. J. Med. Genet.* **14**: 41–8. https://doi.org/10.2478/v10034-011-0046-8

Taheri M, Hashemi-Shahri SM, Hamzehnejadi M, Naderi M, Moazeni-Roodi A, Bahari G, Hashemi M (2012) Lack of association between interleukin-18 -607 C/A gene polymorphism and pulmonary tuberculosis in Zahedan, Southeast Iran. *Prague Med. Rep.* **113**: 16–22. https://doi.org/10.14712/23362936.2015.33

Takada T, Suzuki E, Morohashi K, Gejyo F (2002) Association of single nucleotide polymorphisms in the IL-18 gene with sarcoidosis in a Japanese population. *Tissue Antigens* **60**: 36–42. https://doi.org/10.1034/j.1399-0039.2002.600105.x

Wawrocki S, Druszczynska M, Kowalewicz-Kulbat M, Rudnicka W (2016) Interleukin 18 (IL-18) as a target for immune intervention. *Acta Biochim. Pol.* **63**: https://doi.org/10.18388/abp.2015_1153

Wei Z, Wenhao S, Yuanyuan M, Yang L, Daming Z, Jiangchun X, Jijun J (2017) A single nucleotide polymorphism in the interferon-γ gene (IFNG +874 T/A) is associated with susceptibility to tuberculosis. *Oncotarget* **8**: 50415–50429. https://doi.org/10.18632/oncotarget.17304

Yi Y-X, Han J-B, Zhao L, Fang Y, Zhang Y-F, Zhou G-Y (2015) Tumor necrosis factor alpha gene polymorphism contributes to pulmonary tuberculosis susceptibility: evidence from a meta-analysis. *Int. J. Clin. Exp. Med.* **8**: 20690–20700

Zhou C, Ouyang N, Li QH, Luo SX, He Q, Lei H, Liu Q (2015) The -137G/C single nucleotide polymorphism in IL-18 gene promoter contributes to tuberculosis susceptibility in Chinese Han population. *Infect. Genet. Evol.* **36**: 376–380. https://doi.org/10.1016/j.meegid.2015.10.014

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RESEARCH ARTICLE

# IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB

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## Abstract

### Background

Currently, there are serious limitations in the direct diagnosis of active tuberculosis (ATB). We evaluated the levels of the IL-18/IL-37/IP-10 signalling complex proteins in *Mycobacterium tuberculosis* (*M.tb*)-specific antigen-stimulated QuantiFERON® Gold In-Tube (QFT) cultures and in serum samples from ATB patients, healthy individuals with latent *M.tb* infection (LTBI) and healthy controls (HC) to examine whether combined analyses of these proteins were useful in the differentiation of *M.tb* states.

### Methods

The concentrations of IL-18, IL-18BP, IFN-γ, IL-37 and IP-10 in the serum and QFT supernatants were measured using specific enzyme-linked immunosorbent assay (ELISA) kits. Free IL-18 levels were calculated using the law of mass action.

### Results

Increased concentrations of total and free IL-18, IL-18BP, IFN-γ and IP-10 in the sera of ATB patients were detected. These increases were not counterbalanced by the overproduction of IL-37. Complex co-expression of serum IL-18BP and IL-37, IP-10 and IFN-γ was identified as the highest discriminative biomarker set for the diagnosis of ATB.

### Conclusions

Our results suggest that the IL-18 signalling complex might be exploited by *M. tuberculosis* to expand the clinical manifestations of pulmonary TB. Therefore, direct analysis of the serum components of the IL-18/IL-37 signalling complex and IP-10 may be applicable in designing novel diagnostic tests for ATB.

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## Introduction

Tuberculosis (TB) affects approximately 10 million people causing 2 million deaths annually [1]. Approximately 1/3 of the human population is infected with *Mycobacterium tuberculosis* (*M.tb*), the causative agent of TB, and 5–10% of this population develop active tuberculosis (ATB) disease during their lifetime. The remaining 90–95% of individuals mount an immune response and develop latent tuberculosis infection (LTBI) [2]. TB is predominantly a disease of the lungs and the transmission of *M.tb* bacilli occurs through airborne granulomatous particles released into the air by individuals suffering from pulmonary ATB. Tuberculous granulomas are formed in infected lungs and are aggregates of macrophages surrounded by a lymphocyte marginal zone that encloses the infecting mycobacteria [3]. *M.tb* persists in a dormant state inside macrophages for long periods of time. The immune status of the host's macrophages and T cells and the activation of cytokines, primarily IFN-γ, control the granuloma structure and *M.tb* replication in asymptomatic LTBI. Due to an unpredictable reason, the bacilli reactivate in 5–10% of LTBI subjects, and caseous granulomas develop and initiate a clinical disease and the spread of virulent bacilli in the environment. The risk of progression from LTBI to active TB may be increased by some factors such as HIV infection, chronic renal failure, diabetes, organ transplantation or therapy using tumor necrosis factor-alpha blockers. The identification of *M.tb*-infected individuals and the appropriate treating those who develop ATB and preventing those with an increased risk of TB progression are undoubtedly crucial for effective TB control. Currently, there are limitations in the direct ATB detection via the microscopic visualization of acid-fast bacteria in the sputum and observation of *M.tb* growth in long-term cultures. Interferon-gamma release assays (IGRAs) are used to diagnose LTBI. These tests measure the release of IFN-γ in response to *M.tb*-specific antigens in whole blood cultures. However, IGRAs in combination with tuberculin skin tests are not sufficiently accurate to diagnose ATB [1,4]. To satisfy the demand for rapid and accurate TB diagnostic tests, we performed analyses of several classification algorithms to rank proteins of the IL-18/IL-37/IP-10 signalling complex according to their usefulness in the differentiation of *M.tb* infection states. Progression towards tuberculosis disease correlates with the loss of organization in the granulomas [5]. The immunological and inflammatory environments of granulomas change due to the recirculation of immune cells and the release of cytokines that reach the periphery. Therefore, evaluations of cytokine/chemokine profiles in the blood are promising for the differentiation of infectious states in TB [6–8].

The cytokine IL-18 is implicated in the protective and pathological processes of *M.tb* infection [9–11]. The activity of IL-18 occurs via an IL-18 binding receptor (IL-18R) complex formed by two chains: a ligand-binding alpha chain (IL-18Rα), and a beta chain (IL-18Rβ), which is responsible for the induction of a proinflammatory signal [12]. The formation of an IL-18Rα/IL-18Rβ heterodimer triggers the signalling cascade that leads to activation of the transcription factor NF-κβ [13]. The excessive inflammatory signalling of IL-18 is reduced by a constitutively secreted IL-18 binding protein (IL-18BP), which neutralizes circulating IL-18 to lower free IL-18 compared to total IL-18 levels. Therefore, the production of IFN-γ and other proinflammatory cytokines is reduced [14]. IL-18BP also shows a high affinity for IL-37, which is an inhibitor of the innate inflammatory responses involved in curbing excessive inflammation [15,16]. After binding to IL-18BP, IL-37 subsequently binds IL-18Rβ, which inhibits the proinflammatory activity of IL-18 [17,18]. We performed a combined analysis of the proteins, free and total IL-18, IL-18BP, IL-37, IFN-γ in QFT supernatants and directly in serum samples from pulmonary TB patients and healthy individuals with or without latent *M.tb* infection to identify new markers for the diagnosis of ATB. In parallel with the proteins' analysis, we measured serum IFN-γ-inducible protein 10 (IP-10/CXCL10) levels, a chemokine mediating leukocyte recruitment and activation.

## Materials and methods

### Study population

A total population of 238 *M. bovis Bacillus Calmette-Guerin* (BCG)-vaccinated adults of both genders, 18–81 years of age, was enrolled in the present study. All participants were unrelated Poles who signed a formal written consent for the use of their blood for research purposes. The study protocol complied with the most recent Declaration of Helsinki, and the Ethics Committee of the University in Lodz, Poland approved the protocol. The study cohort included 95 patients with active pulmonary TB (ATB), which was microbiologically confirmed (51) or not confirmed (44) in a triple sputum culture. They were recruited from the Regional Specialised Hospital of Tuberculosis, Lung Diseases and Rehabilitation in Tuszyn, Poland. A full history was taken from all patients, and experienced physicians completed general and clinical examinations. The final diagnosis was based on the clinical symptoms, a chest X-ray image, microscopic and microbiological evaluations of sputum samples and a proper response to anti-tuberculous treatment. Blood samples were taken prior to the start of therapy. Healthy individuals (143) entering the study were classified as *M.tb*-infected (LTBI) (52) or *M.tb*-uninfected (91) based on the interferon-gamma release assay results (QuantiFERON-TB Gold Plus; QFT). None of the healthy volunteers had a history of TB.

The age, sex, BCG vaccination status as well as tuberculin skin test (TST) and QFT results of the study participants are summarized in Table 1. The group of the study comprised 95 patients diagnosed with active pulmonary TB (ATB), microbiologically confirmed (51) or not confirmed (44) by a triple sputum culture, 52 individuals without TB history, who were latently infected with *M.tb* (LTBI), and 91 healthy controls without *M.tb* infection (HC). There were no significant differences between studied groups regarding age or BCG vaccination rate. The proportion of men in the ATB group (56%) was significantly higher than in LTBI (31%) and HC (31%) groups ($p<0.05$). Fifty one (54%) ATB patients had a positive sputum culture for *M.tb*. Forty four (46%) *M.tb* culture negative patients were diagnosed on the basis of clinical manifestations, typical features' on chest radiographs and proper response to anti-tuberculous treatment. Fifty-five % of ATB patients exhibited a positive TST result with an induration diameter of more than 10 mm, whereas a positive QFT result was found in 61% of individuals from this group. Both subgroups of ATB patients were characterized by a similar proportion of TST-positive and QFT-positive results. Nine out of 95 (9%) ATB patients had a history of healed pulmonary TB. Six percent of ATB patients suffered from diabetes or chronic renal failure, whereas cardiovascular or neurological diseases were diagnosed in 15% and 3% patients, respectively.

### Blood samples

A 5-ml volume of venous blood samples was used to prepare the sera and perform a QuantiFERON-TB® Gold Plus test (QFT, Qiagen, Hilden, Germany). Blood was collected in four 1-ml tubes (Nil control, TB antigen-specific 1 (TB1), TB antigen-specific 2 (TB2), Mitogen control), and IFN-γ levels in the supernatants were measured immunoenzymatically after a 24-hour incubation.

### IL-18, IL-18BP, IL-37, IFN-γ, IP-10 estimation and calculation of free IL-18

The concentrations of IL-18, IL-18BP and IL-37 in the sera and QFT cell-free culture supernatants were determined using commercially available specific ELISA kits: Human Total IL-18 DuoSet ELISA (R&D, Minneapolis, USA), Human IL-18BPa DuoSet ELISA (R&D) and Human IL-37/IL-1F7 Duoset ELISA (R&D). IFN-γ and IP-10 concentrations in sera were

**Table 1. Characteristics of the study participants.**

| | ATB | LTBI | HC |
|---|---|---|---|
| N | 95 | 52 | 91 |
| Ethnicity | Caucasians | Caucasians | Caucasians |
| Country of origin | Poland | Poland | Poland |
| Sex, N (%) | | | |
| Men | 53 (56%) | 16 (31%)[a] | 28 (31%)[b] |
| Women | 42 (44%) | 36 (69%) | 63 (69%) |
| Age | | | |
| Median | 51 | 52 | 41 |
| range | 19–81 | 20–81 | 18–75 |
| years (IQR) | 33–63 | 41–56 | 33–52 |
| BCG vaccination, N (%) | 95 (100%) | 52 (100%) | 91 (100%) |
| TST induration diameter (mm) | | | |
| median | 12 | | |
| range | 0–35 | | |
| TST result, N (%) | | n.d | n.d |
| positive | 52 (55%) | | |
| negative | 43 (45%) | | |
| QFT result, N (%) | | | |
| positive | 58 (61%) | 52 (100%) | 0 (0%) |
| negative | 37 (39%) | 0 (0%) | 91 (100%) |
| *M.tb* culture | | | |
| positive | 51 (54%) | n.d | n.d |
| negative | 44 (46%) | | |
| History of healed ATB | 9 (9%) | 0 (100%) | 0 (100%) |

Abbreviations: ATB–active tuberculosis, HC- healthy controls, LTBI–latent *M.tb* infection, BCG–Bacillus Calmette-Guerin, QFT–QuantiFERON TB Gold Plus, TST-tuberculin skin test, IQR—interquartile range, n.d–not determined.

There was a significant difference among studied groups

[a]male LTBI vs ATB ($p<0.05$) and

[b]male HC vs ATB ($p<0.05$) ($\chi^2$ test).

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assessed using Human IFN-γ Duoset ELISA (R&D) and Human IP-10 Duoset ELISA (R&D). The law of mass action was used to calculate the level of free IL-18 [19]. It is known that one IL-18BP molecule binds a single molecule of IL-18, and this interaction has a dissociation constant (Kd) of 0.4 nM. Therefore, the level of free IL-18 was calculated from the equation $x = (-b \pm (b^2-4ac)^{1/2})/2a$, where x is [IL-18free], b is = [IL-18BP]–[IL-18] + Kd, and c is -Kd·IL-18][13,19,20]. The levels of the studied proteins in the QFT supernatants were calculated after subtraction of baseline levels obtained from NIL tube.

## Statistical analyses

Statistical analyses were performed using Statistica 12 PL (Statsoft, Poland). Comparisons of the frequencies were tested using the $\chi^2$ or Fisher's exact test. Differences in the levels of the studied proteins were analysed using the non-parametric Kruskal-Wallis test. Differences with $p < 0.05$ were considered statistically significant.

Target statistical and machine-learning methods were used as implemented in R. 'pROC', 'ROCR' packages as well as custom codes (available upon request) were used in the ROC analysis. For the classical analysis of association between levels of expression of single proteins and

protein ratios logistic regression was used in order to make the comparison of performance of single markers to all available markers (full logistic model) 'fair'. The AUC values were estimated via 5-fold cross-validation and based on at least 500 bootstrap replicates. The Random Forest algorithm was used as implemented in the 'randomForest' package. Pearson's correlation coefficient was used for co-expression analyses. For the network analysis, the custom R code was used (available upon request), and data discretization for the estimation of Renyi divergence was performed using the 'infotheo' and 'equalfreq' options. The t-SNE algorithm was used as implemented in 'Rtsne'. The ordinal elastic-net algorithm was applied as implemented in 'ordinalNet'.

## Results

### Serum IL-18, IL-18BP, IL-37, IFN-γ and IP-10 levels in the studied groups

The concentration of total IL-18 in the sera from the ATB patients (median (Me) 568.1 (IQR 371.9, 980.3) pg/ml) was significantly higher than those found in the LTBI (Me 261.6 (IQR 109.3, 492.3) pg/ml) and HC (Me 283.1 (IQR 177.5, 441.4) pg/ml) groups ($p<0.001$) (Fig 1A).

Total IL-18 levels were increased in both culture-positive (Me 719.5 (IQR 416.3, 1146.0) pg/ml) and culture-negative (Me 444.2 (IQR 305.8, 828.1) pg/ml) ATB patients (data not shown). Similarly to the total IL-18 level, the serum concentration of IL-18BP was significantly higher in ATB patients (Me 43.5 (IQR 31.7, 60.4) ng/ml, culture-positive (Me 44.9 (IQR 32.5, 59.3) ng/ml, culture-negative (Me 43.4 (IQR 27.8, 77.2) ng/ml) than in LTBI (Me 28.8 (IQR 21.1, 42.8) ng/ml; $p<0.001$) or HC (Me 32.7 (IQR 22.7, 43.7) ng/ml; $p<0.001$) groups (Fig 1B).

The serum concentration of free IL-18 calculated from IL-18 and IL-18BP levels was significantly higher in ATB patients (Me 5.5 (IQR 3.0, 10.5) pg/ml; culture-positive (Me 5.5 (IQR 3.7, 10.9) pg/ml, culture-negative (Me 3.6 (IQR 2.1, 8.3) pg/ml) than LTBI (Me 3.6 (IQR 1.7, 5.5)

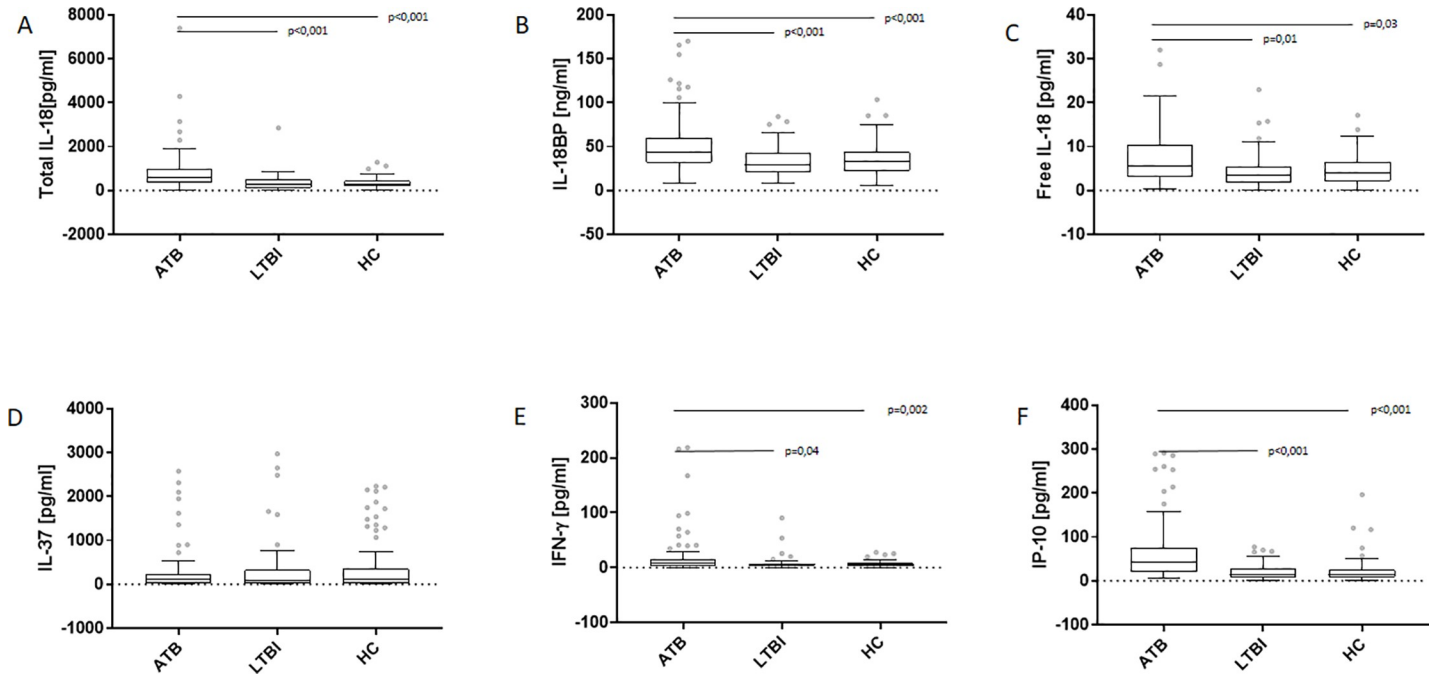


**Fig 1. Serum levels of total and free IL-18, IL-18BP, IL-37, IFN-γ and IP-10 in the groups of the study.** Boxplots with median (horizontal line within the box), interquartile range (box limits), and extremes (whiskers) of serum levels of total IL-18 (A), IL-18BP (B), free IL-18 (C), IL-37 (D), IFN-γ (E) and IP-10 (F) in the groups of patients with active tuberculosis (ATB) and latent *M.tb* infection (LTBI) and healthy controls (HC).

https://doi.org/10.1371/journal.pone.0225556.g001

pg/ml; p = 0.012) or HC (Me 4.0 (IQR 2.1, 6.5) pg/ml; p = 0.036) groups (Fig 1C). In contrast, the serum concentrations of IL-37 were similar among studied groups: ATB (Me 102.6 (IQR 12.6, 236.6) pg/ml, LTBI (Me 78.4 (IQR 23.3, 323.3) pg/ml, HC (Me 103.5 (IQR 9.8, 357.3) pg/ml (Fig 1D). On the other hand, the level of IFN-γ, in the ATB patients' sera was significantly higher (Me 6.7 (IQR 3.5, 15.0) pg/ml; culture-positive (Me 8.8 (IQR 4.8, 20.4) pg/ml, culture-negative (Me 5.7 (IQR 3.2, 12.2) pg/ml) than that observed in the LTBI (Me 5.0 (IQR 3.3, 7.5) pg/ml; p = 0.049) or HC (Me 5.7 (IQR 3.0, 8.4) pg/ml; p = 0.002) individuals (Fig 1E). As shown in Fig 1F, the serum level of IP-10 was significantly higher in the ATB group (Me 43.5 (IQR 19.9, 75.7) pg/ml; culture-positive (Me 49.3 (IQR 27.4, 83.9) pg/ml, culture-negative (Me 35.8 (IQR 13.6, 57.2) pg/ml) than in the groups of LTBI (Me 13.7 (IQR 8.2, 28.0) pg/ml) and HC (Me 14.0 (IQR 7.8, 25.3) pg/ml) ($p<0.001$). There were no significant differences in the serum levels of the total IL-18, IL-18BP, free IL-18, IL-37, IFN-γ and IP-10 between ATB patients with positive or negative QFT and TST results (Fig 2A–2F).

Analysis of the area under the ROC revealed that the full logistic model resulted in an AUC = 0.82 (CI = (0.82; 0.83)) for the HC vs. ATB comparison, an AUC = 0.65 (CI = (0.61; 0.70)) for HC vs. LTBI, and an AUC = 0.79 (CI = (0.73; 0.80)) for LTBI vs. ATB. Table 2 shows the AUCs for individual proteins.

The analysis of dependence between the levels of all proteins revealed certain correlations, that were specific for two groups of the study (IL-18BP and IP-10 as well as IL-18 and IL-18BP for ATB and LTBI) and some relationships specific only for certain study groups (e.g. IL-18 and IL-37 or il-18 and IP-10 for HC, IL-18 and IFN-γ or IFN-γ and IP-10 for ATB, and no correlation specific for LTBI) (Table 3).

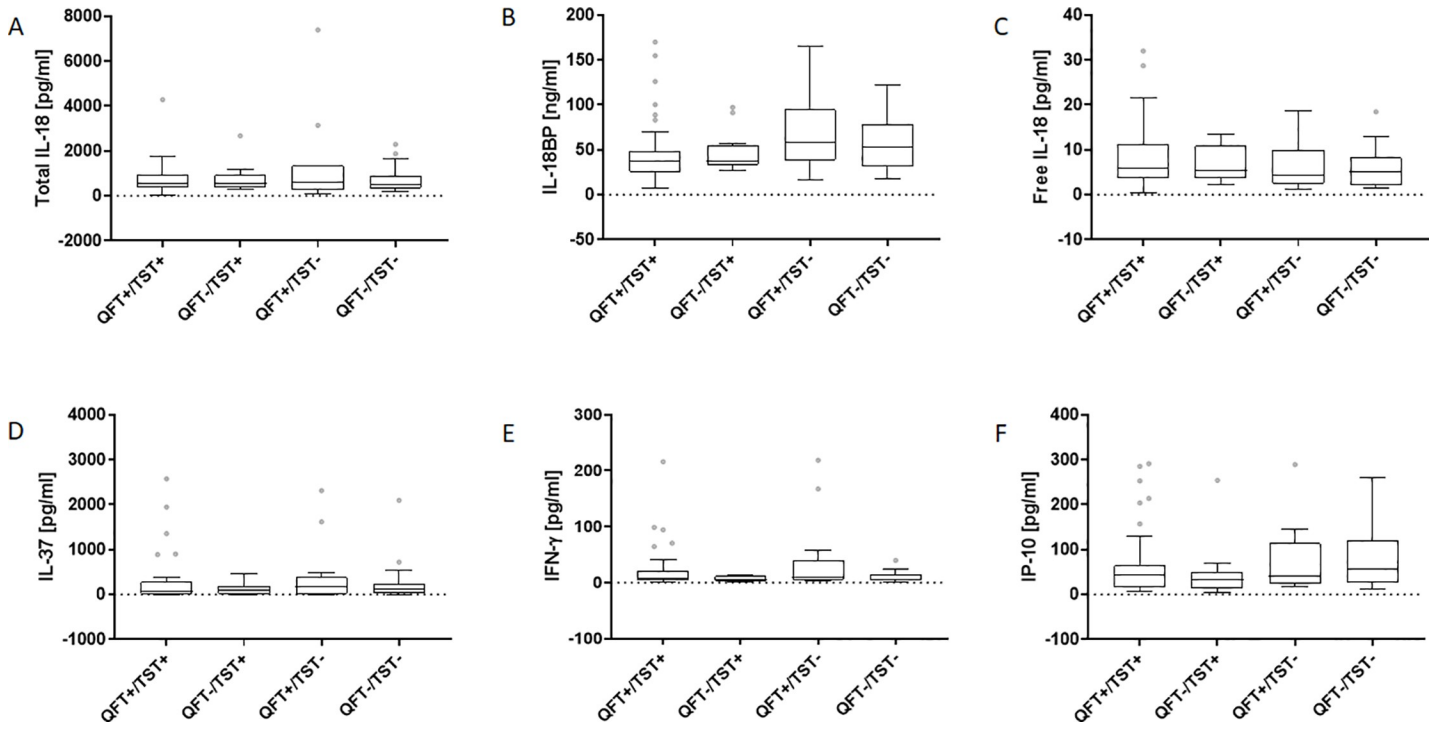


**Fig 2. Serum levels of total IL-18, IL-18BP, free IL-18, IL-37, IP-10 and IFN-γ versus QFT/TST results.** Boxplots with median (horizontal line within the box), interquartile range (box limits), and extremes (whiskers) of serum levels of total IL-18 (A), IL-18BP (B), free IL-18 (C), IL-37 (D), IFN-γ (E) and IP-10 (F) in the group of active tuberculosis patients with positive or negative QuantiFERON (QFT) and tuberculin skin test (TST) results.

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**Table 2. Predictive values (median AUC and 95% CI) of individual protein levels measured in serum.**

| Parameter | HC vs. ATB | HC vs. LTBI | LTBI vs. ATB |
|---|---|---|---|
| | median AUC (95% CI) | | |
| IL-18 | 0.76 (0.76;0.77) | 0.71 (0.65;0.77) | 0.76 (0.75;0.77) |
| IL-18BP | 0.67 (0.66;0.67) | 0.62 (0.56;0.69) | 0.69 (0.68;0.70) |
| IL-37 | 0.59 (0.52;0.59) | 0.76 (0.69;0.82) | 0.64 (0.59;0.69) |
| IFN-γ | 0.57 (0.56;0.58) | 0.61 (0.58;0.67) | 0.52 (0.50;0.56) |
| IP-10 | 0.78 (0.77;0.78) | 0.81 (0.75;0.86) | 0.75 (0.75;0.76) |

ATB–active tuberculosis (n = 95), HC- healthy controls (n = 91), LTBI–latent *M.tb* infection (n = 52)

https://doi.org/10.1371/journal.pone.0225556.t002

## *M.tb* antigens–stimulated IL-18, IL-18BP, IL-37 and IFN-γ levels in QFT supernatants

As shown in Fig 3A, the *M.tb* antigens-stimulated levels of total IL-18 were significantly increased in the patients with ATB (Me 765.5 (IQR 431.6, 1122.0) pg/ml, culture-positive (Me 810.0 (IQR 440.4, 1142.0) pg/ml, culture-negative (Me 734.0 (IQR 403.7, 1131.0) pg/ml) compared to those of the LTBI (Me 445.7 (IQR 29.6, 656.1) pg/ml; $p<0.001$) or the HC group (Me 397.5 (IQR 222.8, 511.6) pg/ml; $p<0.001$). The level of IL-18BP was significantly higher ($p<0.001$) in ATB patients (Me 43.1 (IQR 32.5, 63.8) ng/ml) than HC (Me 32.2 (IQR 18.5, 50.9) ng/ml) (Fig 3B). There were no significant differences in the levels of free IL-18 or IL-37 in the QFT supernatants among studied groups (Fig 3C and 3D). The IFN-γ concentration in QFT supernatants was significantly higher in LTBI (Me 91.8 (IQR 37.5, 262.0) pg/ml) than ATB (Me 39.3 (IQR 13.0, 144.2) pg/ml, culture-positive (Me 49.6 (IQR 18.6, 144.2) pg/ml, culture negative (Me 25.3 (IQR 10.8, 159.0) pg/ml; $p = 0.003$) or HC groups (Me 7.9 (IQR 6.3, 10.6) pg/ml; $p<0.001$) (Fig 3E). However, there was no association between the results of QFT or TST and the levels of IL-18, IL-18BP or IL-37 measured in the QFT supernatants (Fig 4A–4D).

Analysis based on the area under the ROC showed full logistic model of an AUC = 0.91 (CI = (0.90; 0.91)) for the HC vs. ATB comparison, an AUC = 0.98 (CI = (0.95; 0.99)) for HC vs. LTBI, and an AUC = 0.70 (CI = (0.69; 0.71)) for LTBI vs. ATB. Table 4 shows the discriminative power for the levels of individual proteins.

**Table 3. Correlation (r) and p values between protein levels in serum.**

| Parameters | HC | | LTBI | | ATB | |
|---|---|---|---|---|---|---|
| | n = 91 | | n = 52 | | n = 95 | |
| | r | p | r | p | r | p |
| IL-18 ~ IL-18BP | 0.021 | 0.765 | 0.258 | 0.006 | 0.180 | 0.009 |
| IL-18 ~ IL-37 | -0.130 | 0.070 | -0.012 | 0.899 | -0.005 | 0.940 |
| IL-18 ~ IFN-γ | 0.025 | 0.718 | -0.100 | 0.293 | 0.210 | 0.002 |
| IL-18 ~ IP-10 | 0.152 | 0.033 | 0.154 | 0.107 | 0.112 | 0.107 |
| IL-18BP ~ IL-37 | 0.093 | 0.195 | -0.122 | 0.203 | -0.106 | 0.128 |
| IL-18BP ~ IFN-γ | 0.048 | 0.495 | 0.043 | 0.647 | 0.186 | 0.007 |
| IL-18BP ~ IP-10 | 0.100 | 0.163 | 0.188 | 0.049 | 0.239 | <0.001 |
| IL-37 ~ IFN-γ | 0.081 | 0.255 | 0.046 | 0.629 | 0.003 | 0.956 |
| IL-37 ~ IP-10 | 0.018 | 0.796 | -0.089 | 0.350 | 0.042 | 0.546 |
| IFN-γ ~ IP-10 | 0.066 | 0.355 | 0.086 | 0.364 | 0.263 | <0.001 |

ATB–active tuberculosis, HC- healthy controls, LTBI–latent *M.tb* infection

https://doi.org/10.1371/journal.pone.0225556.t003

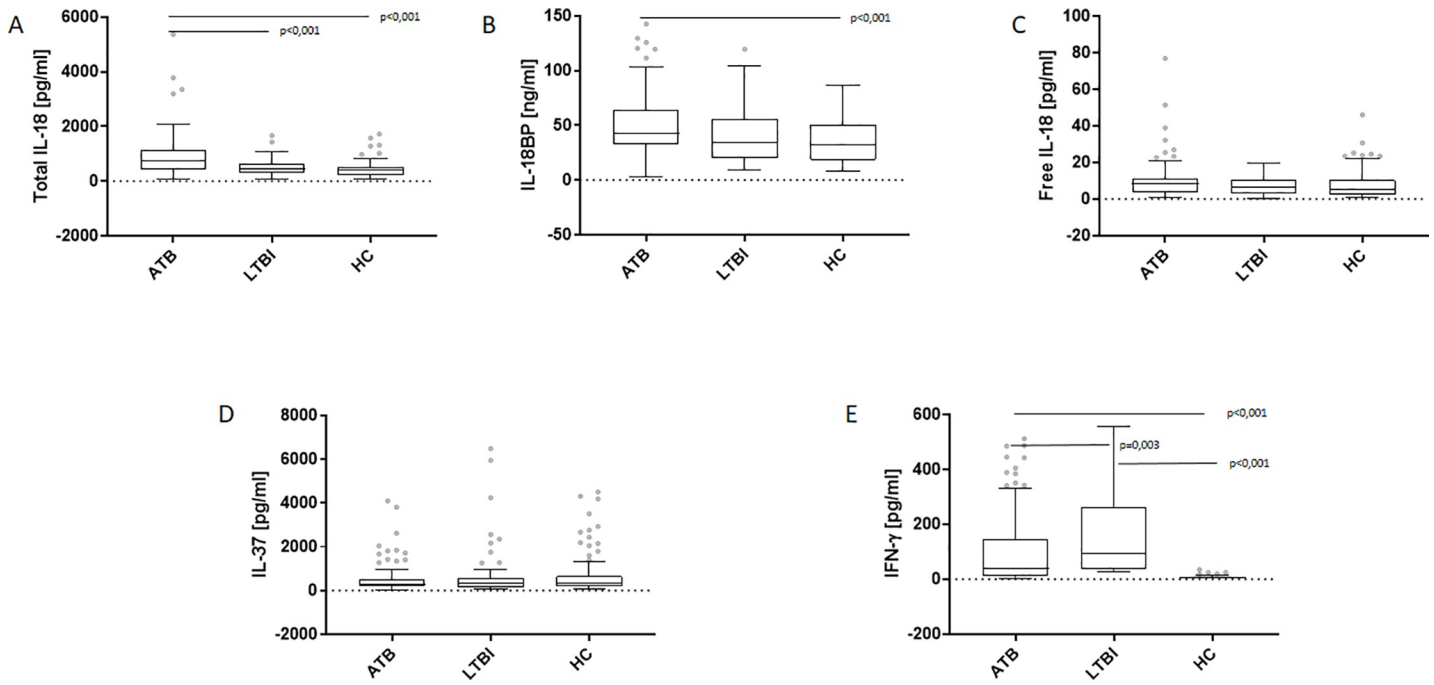


**Fig 3. Total IL-18, IL-18BP, free IL-18, IL-37 and IFN-γ levels in *M.tb* antigens-stimulated QuantiFERON (QFT) supernatants.** Boxplots with median (horizontal line within the box), interquartile range (box limits), and extremes (whiskers) of levels of total IL-18 (A), IL-18BP (B), free IL-18 (C), IL-37 (D) and IFN-γ (E) in *M.tb* antigens-stimulated QFT supernatants in the groups of patients with active tuberculosis (ATB) and latent *M.tb* infection (LTBI) and healthy controls (HC).

https://doi.org/10.1371/journal.pone.0225556.g003

Correlation analyses revealed pairs of proteins that were specific for certain study groups (IL-18 and IFN-γ and IL-18 and IL-37 for HC; IL-18BP and IL-37 for LTBI; and IL-18 and IL-18BP for ATB). A summary of these results is provided in Table 5.

## Protein ratios in the sera and QFT supernatants

The discriminative powers of the ratios of serum and QFT supernatants between any two proteins of the IL-18 signalling complex were analysed. The highest discriminative powers in the sera (1) were IL-18/IL-37 for HC and ATB (AUC = 0.69, CI = (0.68; 0.69)), (2) IL-18/IL-18BP for HC and LTBI (AUC = 0.82, CI = (0.76; 0.88)) and (3) IL-18/IL-18BP for LTBI and ATB (AUC = 0.66, CI = (0.64; 0.67)). On the contrary, the highest discriminative powers in the QFT supernatants were (1) IL-37/IFN-γ for HC and ATB (AUC = 0.75, CI = (0.74; 0.76)), (2) IL-18BP/IFN-γ for HC and LTBI (AUC = 0.91, CI = (0.90; 0.91)), and (3) IL-18/IFN-γ for LTBI and ATB (AUC = 0.72, CI = (0.71; 0.74)).

## Selection of the most informative protein ratios using random forest

An approach based on the random forest, which is a sample classification and feature selection algorithm, returned an importance score for each ratio of protein levels and an optimal number of features to be used in classifications estimated using cross-validation. Even with no statistically significant differences between studied groups, the IL-18, IL-18BP and IFN-γ from QFT supernatants were most informative (in terms of AUC) in the LTBI vs. HC comparison. The IL-18/IL-37, IL-18/IFN-γ, IL-18BP/IFN-γ and IL-37/IFN-γ protein ratios had the highest importance scores, and the levels of IL-18, IL-18BP and IFN-γ and the ratios between IL-18BP/IFN-γ and IL-37/IFN-γ remained informative in joint analyses. IL-18, IL-18BP and IFN-γ were most informative for the ATB vs. HC comparison, and the IL-18/IL-37, IL-18/IFN-γ,

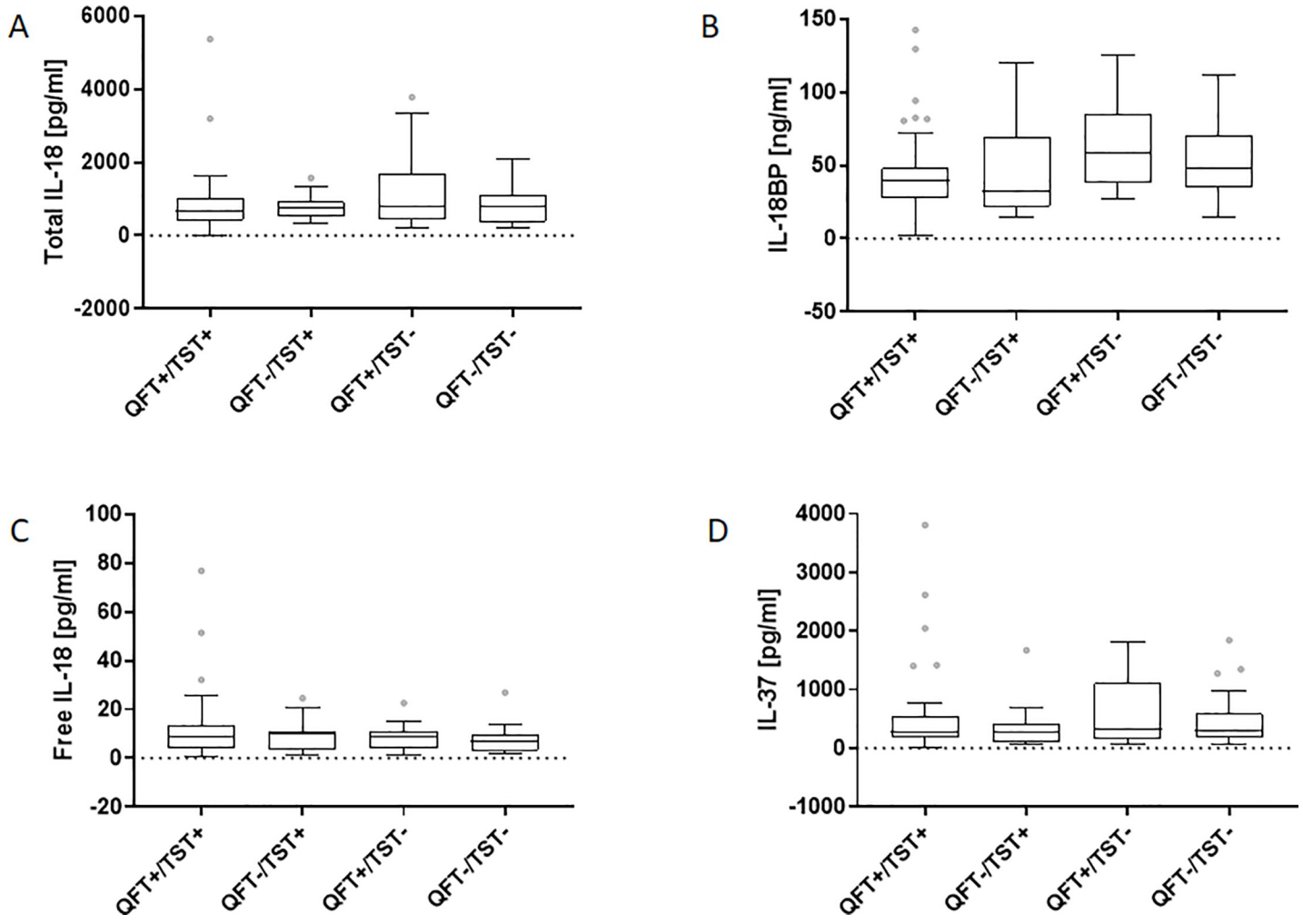


**Fig 4. Levels of total IL-18, IL-18BP, free IL-18 and IL-37 in *M.tb* antigens-stimulated QuantiFERON (QFT) supernatants versus QFT/TST result.** Boxplots with median (horizontal line within the box), interquartile range (box limits), and extremes (whiskers) of levels of total IL-18 (A), IL-18BP (B), free IL-18 (C), IL-37 (D) in *M. tb* antigens-stimulated QFT supernatants from active tuberculosis patients with positive or negative QuantiFERON (QFT) and tuberculin skin test (TST) results.

https://doi.org/10.1371/journal.pone.0225556.g004

IL-18BP/IFN-γ and IL-37/IFN-γ protein ratios had the highest importance scores. The levels of IL-18, IL-18BP and IFN-γ and the ratios between IL-18/IL-37 and IL-37/IFN-γ remained informative in the joint analyses. IL-18 and IFN-γ were most informative for the LTBI vs. ATB comparison, and the IL-18/IL-18BP, IL-18/IL-37, IL-18/IFN-γ, IL-18BP/IL-37 and IL-18BP/

**Table 4. Predictive values (median AUC and 95% CI) of individual protein levels in QFT supernatants.**

| Parameter | HC vs. ATB | HC vs. LTBI | LTBI vs. ATB |
|---|---|---|---|
| | median AUC (95% CI) | | |
| IL-18 | 0.76 (0.76;0.77) | 0.51 (0.50;0.54) | 0.71 (0.69;0.72) |
| IL-18BP | 0.64 (0.64;0.65) | 0.48 (0.45;0.55) | 0.58 (0.56;0.59) |
| IL-37 | 0.54 (0.50;0.58) | 0.75 (0.69;0.83) | 0.59 (0.55;0.63) |
| IFN-γ | 0.83 (0.83;0.84) | 0.99 (0.99;0.99) | 0.58 (0.55;0.62) |

ATB–active tuberculosis (n = 95), HC- healthy controls (n = 91), LTBI–latent *M.tb* infection (n = 52)

https://doi.org/10.1371/journal.pone.0225556.t004

**Table 5. Correlation (r) and p-values between protein levels in QFT supernatants.**

| Parameters | HC | | LTBI | | ATB | |
|---|---|---|---|---|---|---|
| | n = 91 | | n = 52 | | n = 95 | |
| | r | p | r | p | r | p |
| IL-18 ~ IL-18BP | -0.164 | 0.021 | -0.006 | 0.943 | 0.148 | 0.033 |
| IL-18 ~ IL-37 | -0.136 | 0.056 | -0.006 | 0.949 | -0.055 | 0.427 |
| IL-18 ~ IFN-γ | -0.139 | 0.051 | -0.075 | 0.430 | 0.011 | 0.867 |
| IL-18BP ~ IL-37 | 0.042 | 0.550 | -0.252 | 0.008 | -0.053 | 0.446 |
| IL-18BP ~ IFN-γ | 0.023 | 0.742 | -0.020 | 0.831 | 0.016 | 0.816 |
| IL-37 ~ IFN-γ | 0.257 | <0.001 | -0.092 | 0.335 | 0.023 | 0.733 |

ATB–active tuberculosis, HC- healthy controls, LTBI–latent *M.tb* infection

https://doi.org/10.1371/journal.pone.0225556.t005

IFN-γ protein ratios had the highest importance scores. The levels of IL-18, IL-18BP and IFN-γ and the IL-18/IL-18BP, IL-18/IL-37, IL-18/IFN-γ and IL-18BP/IFN-γ protein ratios remained informative in the joint analyses.

IL-18 and IL-37 were the most informative serum markers for the LTBI vs. HC comparison. The protein ratios IL-18/IFN-γ, IL-18BP/IFN-γ, and IL-18BP/IP-10 had the highest importance scores, and the levels of IL-18, IL-18BP and IFN-γ and the IL-18/IFN-γ, IL-18BP/IL-37, IL-18BP/IFN-γ and IL-18BP/IP-10 protein ratios remained informative in the joint analyses. IL-18 and IP-10 were most informative for ATB vs. HC comparison, and all of the ratios, except IL-18/IP-10 and IL-37/IFN-γ, were informative. The levels of IL-18 and IP-10 had the highest discriminative power in the joint analysis. IL-18, IL-18BP and IP-10 were the most informative for the LTBI vs. ATB comparison, and the IL-18/IL-18BP, IL-18/IL-37, IL-18/IFN-γ and IL-18BP/IP-10 ratios had highest importance scores. The levels of IL-18, IL-18BP and IP-10 and the IL-18/IL-18BP, IL-18/IL-37, IL-18/IFN-γ and IL-18BP/IP-10 ratios remained informative in the joint analysis. The AUC values are summarized in Table 6.

The three-class comparison of the markers quantified in QFT supernatants revealed that all single protein levels were informative with a multi-class AUC = 0.7728. The IL-18/IFN-γ, IL-18BP/IFN-γ and IL-37/IFN-γ protein ratios had the highest importance scores with an AUC = 0.6026. IL-18 and IFN-γ and the IL-18/IFN-γ and IL-18BP/IFN-γ ratios were the most informative in the joint model with an AUC = 0.772.

Only IL-18 and IP-10 in the serum were informative with a multi-class AUC = 0.6507. All of the ratios, except IL-37/IFN-γ, were informative with an AUC = 0.6192. IL-18, IL-18BP and IP-10 as wells as the IL-18/IL-37, IL-18/IFN-γ and IL-18BP/IP-10 protein ratios were informative in the joint model with an AUC = 0.657.

**Table 6. AUC values for selected decision trees.**

| Comparison | QFT supernatant | | | Serum | | |
|---|---|---|---|---|---|---|
| | expression | ratio | both | expression | ratio | both |
| HC vs. LTBI | 0.997 | 0.940 | 0.976 | 0.561 | 0.501 | 0.502 |
| HC vs. ATB | 0.883 | 0.722 | 0.906 | 0.796 | 0.733 | 0.798 |
| LTBI vs. ATB | 0.751 | 0.722 | 0.773 | 0.758 | 0.661 | 0.739 |

ATB–active tuberculosis (n = 95), HC- healthy controls (n = 91), LTBI–latent *M.tb* infection (n = 52)

For each case, the first value (expression) refers to the model with levels of single proteins only, the second value (ratio) to the ratios between protein levels and the third value (both) to the joint model.

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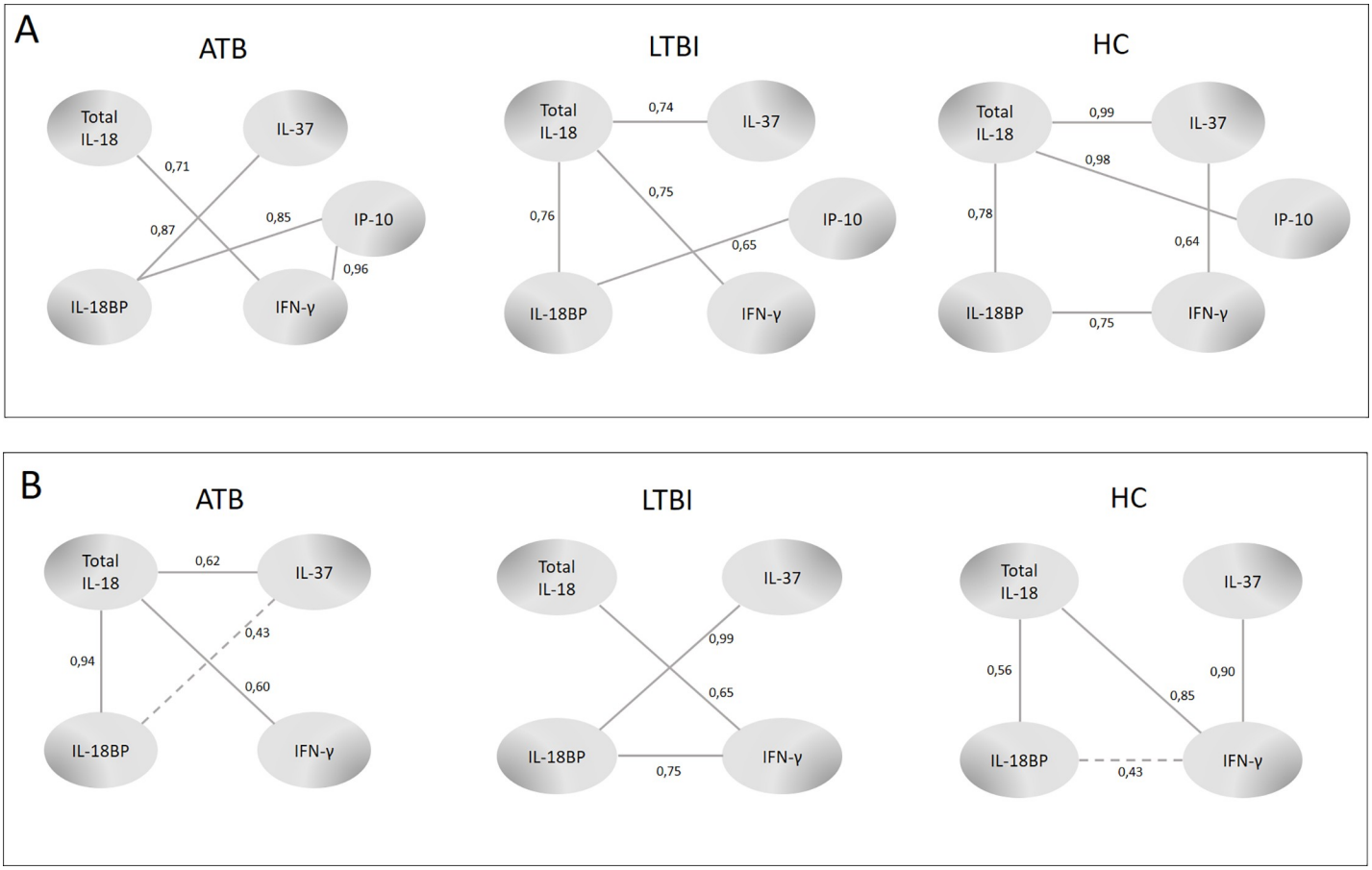


**Fig 5.** Network patterns among studied proteins measured in serum (A) and QFT supernatants (B) from pulmonary TB patients (ATB), latently infected subjects (LTBI) and non-infected healthy controls (HC) The numbers, all between 0 and 1, correspond to the 'likelihood' of observing a given co-expression in a network generated from a random subset of (at least 80% of) individuals in the group of interest.

https://doi.org/10.1371/journal.pone.0225556.g005

## Variability of expression and co-expression levels

To shed some further light on the issue of insufficient discriminative power of single protein levels and protein ratios, we aim to perform a deeper study of the variability and co-expression of the selected markers. In addition to the differential expression between the study groups, IFN-γ levels had significantly different variance between HC and ATB and HC and LTBI in QFT supernatants ($p<10^{-10}$ in both cases). IL-18 was differentially variable between HC and LTBI in QFT supernatants ($p\sim10^{-4}$). IL-18, IL-18BP, IFN-γ and IP-10 in the serum samples were differentially variable between HC and LTBI, and IL-18 and IP-10 were differentially variable between LTBI and ATB.

We used the co-expression network-building method introduced by Hartmann et al. [21] and found that pairs of proteins were stably co-expressed in one study group and co-expressed in a varying fashion in another study group, which is a phenomenon that we call differential co-expression. The co-expression networks generated using this approach are presented in Fig 5. The relationships between (a) IP-10 and IFN-γ as well as between IL-18BP and IL-37 in the serum samples were most robust in the ATB group, (b) IL-18BP and IP-10 as well as IL-18 and IFN-γ were more robust in the ATB or LTBI groups than the HC group, and (c) IL-18 and IP-10, IL-18BP and IFN-γ as well as between IL-37 and IFN-γ were most robust in the HC group

**Table 7. Results for ordinal regression (elastic net)–coefficients.** All available predictors (5 levels of proteins and 10 ratios between these proteins in serum together with 4 protein levels and 6 ratios between proteins in QFT supernatants) were used to model the distribution of an ordinal random variable, Y, which equalled 1 for HC, 2 for LTBI and 3 for ATB. The non-zero coefficients are regarded as being informative of the (conditional) distribution of Y.

| Parameters | Coefficients (95% CI) | |
|---|---|---|
| | logit(P[Y = 1\|Y> = 1]) | logit(P[Y = 2\|Y> = 2]) |
| Serum | | |
| (Intercept) | 5.007 (2.320; 5.241) | 3.745 (2.144; 3.779) |
| IL-18 | -0.115 (-0.232; 0) | -0.073 (-0.232; 0) |
| IL-18BP | -0.237 (-0.311; -0.082) | -0.237 (-0.317; -0.093) |
| IL-37 | 0.129 (0; 0.047) | 0.046 (0; 0.002) |
| IFN-γ | -0.848 (-0.633; -0,020) | -0.848 (-0.633; -0.020) |
| IP-10 | -0.113 (-0.149; -0.062) | -0.157 (-0.175; -0.079) |
| IL-18/IL-18BP | -0.545 (-0.811; 0) | -1.025 (-0.858; 0) |
| IL-18/IL-37 | 0.129 (0;0.015) | 0.129 (0; 0.015) |
| IL-18/IFN-γ | -0.051 (-0.12; 0) | 0.079 (-0.007; 0.056) |
| IL-18/IP-10 | 0 (0; 0.154) | 0 (0; 0.088) |
| IL-18BP/IL-37 | 0 (-0.019; 0) | -0.153 (-0.081; 0) |
| IL-18BP/IFN-γ | 0 (-0.155; 0) | 0 (-0.209; 0) |
| IL-18BP/IP-10 | 0.222 (0.150; 0.431) | -0.005 (0; 0.430) |
| IL-37/IFN-γ | 0 (0; 0.023) | 0 (-0.007; 0.021) |
| IL-37/IP-10 | 0 (-0.034; 0) | -0.200 (-0.063; 0) |
| IFN-γ/IP-10 | 1.300 (0; 0.851) | 2.427 (0; 1.589) |
| QFT supernatant | | |
| IL-18 | -0.371 (-0.419; -0.063) | 0 (-0.123; 0) |
| IL-18BP | -0.097 (-0.540; -0.217) | -0.028 (-0.125; 0) |
| IL-37 | -0.086 (-0.067; 0) | 0.011 (-0.032; 0.004) |
| IFN-γ | -0.981 (-0.478; -0.153) | -0.027 (-0.027; 0) |
| IL-18/IL-18BP | 0.433 (0; 0) | -0.106 (-0.252; 0) |
| IL-18/IL-37 | 0 (-0.028; 0) | 0 (-0.035; 0) |
| IL-18/IFN-γ | 0.301 (0; 0.602) | -0.363 (-0.418; -0.021) |
| IL-18BP /IL-37 | -0.543 (-0.514; -0.074) | 0.089 (-0.022; 0.232) |
| IL-18BP/IFN-γ | 0.266 (0.411; 1. 116) | -0.659 (-0.947; -0.247) |
| IL-37/IFN-γ | 0.047 (0.031; 0.211) | -0.009 (0; 0.049) |

The CI's were estimated via boostrap procedure based on 300 repetitions with the size of the subsample equal to 80% of the original sample. This approach is proposed due to lack of closed form formulas for the distributions of the coefficients. Due to differences in sample size, there are instances where the coefficient lies outside of the CI indicating lack of robustness.

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(Fig 5A). The relationship between (a) IL-18 and IL-37 in QFT supernatants was most robust in the ATB groups, (b) IL-18BP and IL-37 were more robust in the ATB or LTBI group than in the HC group, and (c) IL-37 and IFN-γ were most robust in the HC group (Fig 5B).

## Ordinal Elastic Net for the detection of protein levels and ratios predictive of the transition from HC through LTBI to ATB

The feature selection method based on ordinal regression with elastic net penalty revealed several important predictors in the levels and ratios of the sera and QFT supernatant proteins. All available predictors (5 levels of proteins and 10 ratios between these proteins in serum together with 4 protein levels and 6 ratios between proteins in QFT supernatants) were used to model

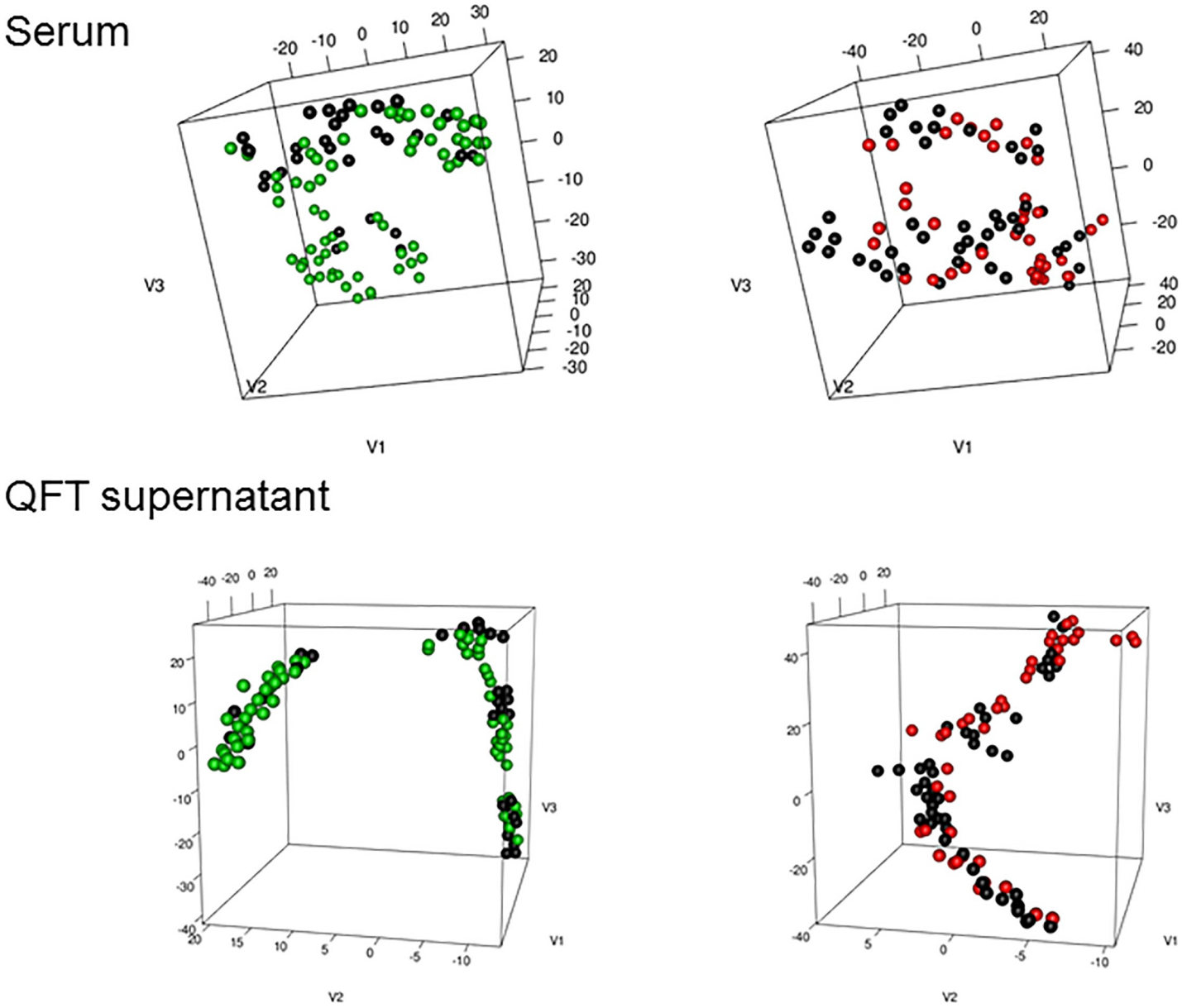


**Fig 6.** Results of the t-SNE algoritm on serum (top) and QFT supernatant (bottom). IGRA negative samples are in the left and IGRA positive in the right panel. ATB cases are in black, LTBI in red and HC in green.

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the distribution of a random variable, Y, which equalled 1 for HC, 2 for LTBI and 3 for ATB. The performance of the model is presented in Table 7. Notably, the expression levels of all proteins in the serum and QFT supernatants were informative predictors of the infection status. However, a number of non-informative expression ratios were found: IL-18/IP-10, IL-18BP/IFN-γ, and IL-37/IFN-γ in the serum and IL-18/IL-37 in QFT supernatants.

## Unsupervised dimension reduction

We also asked whether the molecular signature based on the expression of the studied proteins and ratios may be used to define a meaningful partition of our cohort. We used an

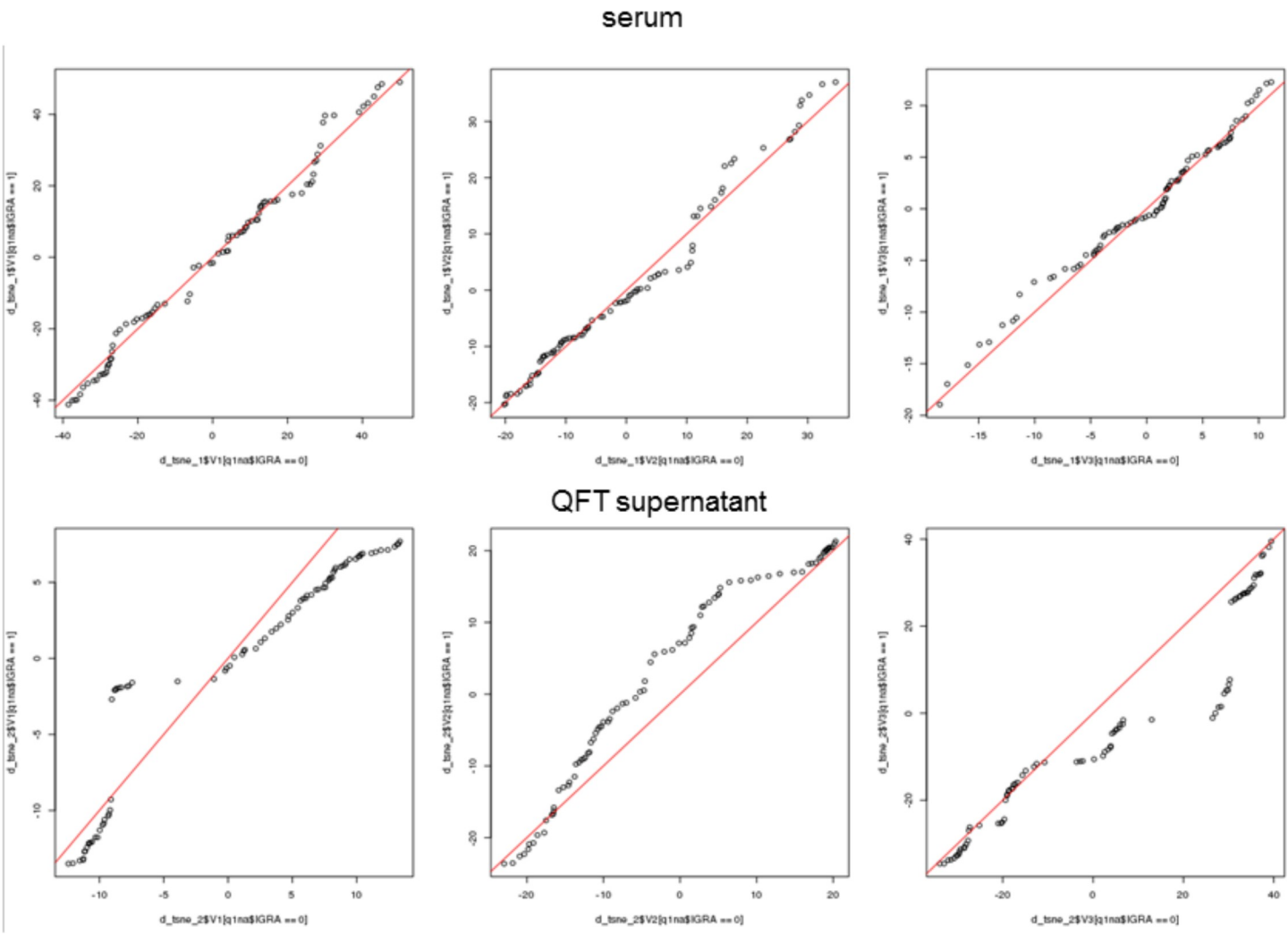


**Fig 7. qq-plots for the components of the t-SNE projection.** The top panel corresponds to the serum and the bottom to the QFT supernatant samples. The leftmost plot in each panel corresponds to the first coordinate of the embedding, the central plot to the second and the rightmost to the third coordinate of the embedding. The x-axis corresponds to IGRA negative and the y-axis to the IGRA positive samples. The red line is the diagonal. In the top panel (serum) the distribution of the consecutive components of the embedding is much more similar between IGRA positive and negative individuals, then in the bottom panel (QFT supernatants).

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unsupervised dimension reduction technique, t-SNE, which is a non-linear alternative to standard Principal Component Analysis.

First, we performed t-SNE on serum and QFT supernatants separately, with no prior information on the studied individuals (Fig 6). For the serum samples, there was no clear clustering or separation between the studied groups. At the same time, there was a separation between the IGRA-negative and IGRA-positive samples for the QFT supernatants as confirmed in the analysis of the similarity of distributions of the three components (derived from t-SNE) between the IGRA-positive and -negative groups (see qq-plots in Fig 7). Then, we used the t-SNE conditionally on the IGRA result. In other words, we performed the dimension reduction separately for the IGRA-positive and -negative individuals. This prior information allowed us to identify two clusters for the IGRA-positive and IGRA-negative samples in the serum with a clearer separation between the ATB and HC groups than between the ATB and LTBI groups (only the active TB and HC could have a negative IGRA in our study) (Fig 6). For QFT

supernatant samples and IGRA-negative samples, we separated a group of healthy controls from the two clusters of the mixed samples (ATB and HC), and we noticed that the likelihood of the sample being classified as LTBI rather than ATB increased for IGRA-positive individuals with the increase in the value of the third projection of the embedding (Fig 6, Fig 7).

## Discussion

We estimated whether the levels of individual proteins of the IL-18 signalling complex i.e. total and free IL-18, IL-18BP, IL-37, IFN-γ and the IP-10 chemokine, and their mutual relationships and ratios, were useful as auxiliary biomarkers of ATB. The analyses showed a significant increase in the serum levels of total and free IL-18, IL-18BP, IFN-γ and IP-10 in ATB patients compared to LTBI or HC individuals. In contrast, a slightly lower serum concentration of the anti-inflammatory IL-37 was measured in ATB than in LTBI or HC groups [16]. This observation indicates a significant loss of balance in the range of the IL-18 signaling complex in ATB. An elevated serum IL-18 concentration in ATB had been previously demonstrated [22–25]. However, our study analysed the IL-18 signalling complex in a wider extent, by evaluating the total and free IL-18, IL-18BP, IL-37, IFN-γ and IP-10 chemokine, in two *M.tb* infection states, which to the best of our knowledge, had not been done previously. We performed statistical analyses of several classification algorithms to rank the measured proteins for their usefulness in the differentiation of *M.tb* infection states using unstimulated serum samples and *M.tb* antigen-stimulated QuantiFERON culture supernatants. Our data showed that individual serum proteins, except IL-37, were able to discriminate between ATB and LTBI in both groups of positive and negative QFT tests. However, the highest discriminative biomarker set was a complex co-expression of serum IL-18BP and IL-37 and IP-10 and IFN-γ, which may be useful in the rapid differentiation between ATB patients and LTBI individuals. Our data are consistent with the opinion that a complex biomarker panel is more robust than single markers for TB screening [5–7,26]. The set of seven serum biosignatures, comprised of apolipoprotein-A1, CRP, complement factor H, IFN-γ, IP-10, serum amyloid A and transthyretin, and a panel of five other serum biomarkers, including IFN-γ, IL-6, IL-18, CRP and MIG, showed potential in screening for TB in African countries endemic for HIV infection [8,27].

Our data suggest that the unstimulated biomarker performance is a better approach to evaluate the systemic manifestation of active TB compared to *M.tb*-stimulated marker expression. In contrast to the five individual serum proteins of the IL-18 signalling complex that were able to discriminate between the ATB and LTBI groups, only total IL-18 and IFN-γ showed significantly different values in QFT supernatants from ATB patients and LTBI individuals. Other results demonstrated that the cytokine ratios might provide specific and sensitive TB indicators [28–30]. A potential role of the IFN-γ/IL-2 ratio in the diagnosis of extrapulmonary TB was reported [31]. The IFN-γ/IL-4 and IL-4δ2/IL-4 mRNA ratios may serve as valuable markers for TB susceptibility or resistance [31,32]. La Manna et al. indicated that 14 analytes (IL-2, IP-10, IFN-γ, MIG, SCF, b-NGF, IL-12-p40, TRAIL, IL2Ra, MIF, TNF-β, IL-3, IFN-α 2, and LIF) allowed discriminating between ATB and non-TB groups [33].

The present study revealed significantly higher levels of circulating IFN-γ in ATB patients than in the LTBI or HC groups. In contrast, the IFN-γ concentration in *M.tb*-stimulated QFT cultures was lower in ATB compared to LTBI subjects. This difference suggests that the activation of an antimycobacterial immune response during ATB occurs concomitantly with the signs of immune depression [34]. However, it cannot be excluded that the most *M.tb*-reactive T cells are redistributed from the periphery to the site of infection, and consequently, only less responsive *M.tb*-specific T cells remain in the circulation. The elevated levels of serum IP-10 in ATB patients support this hypothesis because this chemokine recruits Th1 lymphocytes and

NK cells toward infected areas [35]. We previously reported that circulating leukocytes from healthy BCG-vaccinated individuals become effector cells producing IFN-γ upon stimulation with mycobacterial antigens [36]. The proportion of T $CD4^+$ Th1 cells synthesizing IFN-γ in these cultures significantly exceeded the proportion of T $CD8^+$ cells and NK cells. The IL-18 enhanced IFN-γ production by naïve rather than memory $CD4^+$ Th1 cells [37]. To perform this function, $CD4^+$ Th1 cells recognized *M.tb* antigens that were presented via major histocompatibility complex (MHC) class II molecules at the surface of dendritic cells. It is possible that antigen presentation to $CD4^+$ Th1 cells in active TB might not be optimal for the IFN-γ response in QFT cultures. With this in mind, the search for novel antigens, other than those used in IFN-γ-release assays (ESAT-6/CFP-10/TB7.7), was undertaken by Chegou et al. [38]. Alternatively, we also speculate that new cell culture models that mimic the microenvironment of human lung tissue [39] may be used for the development of a robust immunodiagnosis of TB. In regards to such a supposition, the co-expression of the proteins of the IL-18/IL37 signalling complex and IP-10 should be further analysed in patients with pulmonary disease including those with pulmonary disease other than TB.

## Conclusion

Our results show that the IL-18 signalling complex may be exploited by *M. tuberculosis* to expand the clinical manifestation of pulmonary TB. Therefore, direct analysis of serum components of the IL-18/IL-37 signalling complex and IP-10 may be applicable in designing novel rapid screening tests for pulmonary TB.

## Supporting information

**S1 File. Raw data serum.**
(XLSX)

**S2 File. Raw data QFT.**
(XLSX)

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**Writing – original draft:** Sebastian Wawrocki, Michal Seweryn, Wieslawa Rudnicka, Magdalena Druszczynska.

## References


1. Organization WHO. Global Tuberculosis Report 2018. 2018; Available: https://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf
2. Takenami I, Loureiro C, Machado A, Emodi K, Riley LW, Arruda S. Blood cells and interferon-gamma levels correlation in latent tuberculosis infection. ISRN Pulmonol. 2013; 2013: 1–8. https://doi.org/10.1155/2013/256148 PMID: 24040564
3. Russell DG, Cardona P-J, Kim M-J, Allain S, Altare F. Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol. 2009; 10: 943–948. https://doi.org/10.1038/ni.1781 PMID: 19692995
4. Wlodarczyk M, Rudnicka W, Janiszewska-Drobinska B, Kielnierowski G, Kowalewicz-Kulbat M, Fol M, et al. Interferon-gamma assay in combination with tuberculin skin test are insufficient for the diagnosis of culture-negative pulmonary tuberculosis. PLoS One. Public Library of Science; 2014; 9: e107208. https://doi.org/10.1371/journal.pone.0107208 PMID: 25221998
5. Kunnath-Velayudhan S, Gennaro ML. Immunodiagnosis of tuberculosis: a dynamic view of biomarker discovery. Clin Microbiol Rev. American Society for Microbiology (ASM); 2011; 24: 792–805. https://doi.org/10.1128/CMR.00014-11 PMID: 21976609
6. Weiner J, Kaufmann SHE. High-throughput and computational approaches for diagnostic and prognostic host tuberculosis biomarkers. Int J Infect Dis. 2017; 56: 258–262. https://doi.org/10.1016/j.ijid.2016.10.017 PMID: 27836792
7. Awoniyi DO, Teuchert A, Sutherland JS, Mayanja-Kizza H, Howe R, Mihret A, et al. Evaluation of cytokine responses against novel Mtb antigens as diagnostic markers for TB disease. J Infect. 2016; 73: 219–230. https://doi.org/10.1016/j.jinf.2016.04.036 PMID: 27311746
8. Farr K, Ravindran R, Strnad L, Chang E, Chaisson LH, Yoon C, et al. Diagnostic performance of blood inflammatory markers for tuberculosis screening in people living with HIV. Ivanyi J, editor. PLoS One. 2018; 13: e0206119. https://doi.org/10.1371/journal.pone.0206119 PMID: 30352099
9. Barksby HE, Lea SR, Preshaw PM, Taylor JJ. The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. Clin Exp Immunol. 2007; 149: 217–225. https://doi.org/10.1111/j.1365-2249.2007.03441.x PMID: 17590166
10. Dima E, Koltsida O, Katsaounou P, Vakali S, Koutsoukou A, Koulouris NG, et al. Implication of Interleukin (IL)-18 in the pathogenesis of chronic obstructive pulmonary disease (COPD). Cytokine. 2015; 74: 313–317. https://doi.org/10.1016/j.cyto.2015.04.008 PMID: 25922275
11. Schneider BE, Korbel D, Hagens K, Koch M, Raupach B, Enders J, et al. A role for IL-18 in protective immunity against *Mycobacterium tuberculosis*. Eur J Immunol. 2010; 40: 396–405. https://doi.org/10.1002/eji.200939583 PMID: 19950174
12. Dinarello CA. IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol. 1999; 103: 11–24. Available: http://www.ncbi.nlm.nih.gov/pubmed/9893178 https://doi.org/10.1016/s0091-6749(99)70518-x PMID: 9893178
13. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 Binding Protein. Front Immunol. 2013; 4: 289. https://doi.org/10.3389/fimmu.2013.00289 PMID: 24115947
14. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin -18 regulates bothTH1 and TH2 responses. Annu Rev Immunol. 2001; 19: 423–474. https://doi.org/10.1146/annurev.immunol.19.1.423 PMID: 11244043
15. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol. NIH Public Access; 2010; 11: 1014–22. https://doi.org/10.1038/ni.1944 PMID: 20935647
16. Dinarello CA, Nold-Petry C, Nold M, Fujita M, Li S, Kim S, et al. Suppression of innate inflammation and immunity by interleukin-37. Eur J Immunol. 2016; 46: 1067–1081. https://doi.org/10.1002/eji.201545828 PMID: 27060871
17. Banda NK, Vondracek A, Kraus D, Dinarello CA, Kim S-H, Bendele A, et al. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. J Immunol. 2003; 170: 2100–5. Available: http://www.ncbi.nlm.nih.gov/pubmed/12574381 https://doi.org/10.4049/jimmunol.170.4.2100 PMID: 12574381

18. Bufler P, Azam T, Gamboni-Robertson F, Reznikov LL, Kumar S, Dinarello CA, et al. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. Proc Natl Acad Sci U S A. 2002; 99: 13723–8. https://doi.org/10.1073/pnas.212519099 PMID: 12381835

19. Migliorini P, Anzilotti C, Pratesi F, Quattroni P, Bargagna M, Dinarello CA, et al. Serum and urinary levels of IL-18 and its inhibitor IL-18BP in systemic lupus erythematosus. Eur Cytokine Netw. 2010; 21: 264–71. https://doi.org/10.1684/ecn.2010.0210 PMID: 21126942

20. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci U S A. National Academy of Sciences; 2000; 97: 1190–5. Available: http://www.ncbi.nlm.nih.gov/pubmed/10655506

21. Hartmann K, Seweryn M, Handleman SK, Rempała GA, Sadee W. Non-linear interactions between candidate genes of myocardial infarction revealed in mRNA expression profiles. BMC Genomics. 2016; 17: 738. https://doi.org/10.1186/s12864-016-3075-6 PMID: 27640124

22. Morosini M, Meloni F, Marone Bianco A, Paschetto E, Uccelli M, Pozzi E, et al. The assessment of IFN-gamma and its regulatory cytokines in the plasma and bronchoalveolar lavage fluid of patients with active pulmonary tuberculosis. Int J Tuberc Lung Dis. 2003; 7: 994–1000. http://www.ncbi.nlm.nih.gov/pubmed/14552571 PMID: 14552571

23. Yamada G, Shijubo N, Shigehara K, Okamura H, Kurimoto M, Abe S. Increased levels of circulating interleukin-18 in patients with advanced tuberculosis. Am J Respir Crit Care Med. 2000; 161: 1786–9. https://doi.org/10.1164/ajrccm.161.6.9911054 PMID: 10852745

24. Vankayalapati R, Wizel B, Weis SE, Samten B, Girard WM, Barnes PF. Production of Interleukin-18 in human tuberculosis. J Infect Dis. 2000; 182: 234–239. https://doi.org/10.1086/315656 PMID: 10882602

25. El-Masry S, Lotfy M, Nasif W, El-Kady I, Al-Badrawy M. Elevated serum level of interleukin (IL)-18, interferon (IFN)-γ and soluble fas in patients with pulmonary complications in Tuberculosis. Acta Microbiol Immunol Hung. 2007; 54: 65–77. https://doi.org/10.1556/AMicr.54.2007.1.7 PMID: 17523393

26. Won E-J, Choi J-H, Cho Y-N, Jin H-M, Kee HJ, Park Y-W, et al. Biomarkers for discrimination between latent tuberculosis infection and active tuberculosis disease. J Infect. 2017; 74: 281–293. https://doi.org/10.1016/j.jinf.2016.11.010 PMID: 27871809

27. Chegou NN, Sutherland JS, Malherbe S, Crampin AC, Corstjens PLAM, Geluk A, et al. Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. Thorax. 2016; 71: 785–794. https://doi.org/10.1136/thoraxjnl-2015-207999 PMID: 27146200

28. Joshi L, Ponnana M, Sivangala R, Chelluri LK, Nallari P, Penmetsa S, et al. Evaluation of TNF-α, IL-10 and IL-6 Cytokine Production and their correlation with genotype variants amongst tuberculosis patients and their household contacts. Subbian S, editor. PLoS One. 2015; 10: e0137727. https://doi.org/10.1371/journal.pone.0137727 PMID: 26359865

29. Sun Q, Wei W, Sha W. Potential role for *Mycobacterium tuberculosis* specific IL-2 and IFN-γ responses in discriminating between latent infection and active disease after long-term stimulation. Cao C, editor. PLoS One. 2016; 11: e0166501. https://doi.org/10.1371/journal.pone.0166501 PMID: 28033330

30. Suter-Riniker F, Berger A, Mayor D, Bittel P, Iseli P, Bodmer T. Clinical significance of interleukin-2/gamma interferon ratios in *Mycobacterium tuberculosis*-specific T-cell signatures. Clin Vaccine Immunol. 2011; 18: 1395–6. https://doi.org/10.1128/CVI.05013-11 PMID: 21632888

31. Goyal N, Kashyap B, Kaur IR. Significance of IFN-γ/IL-2 ratio as a circulating diagnostic biomarker in extrapulmonary tuberculosis. Scand J Immunol. 2016; 83: 338–344. https://doi.org/10.1111/sji.12424 PMID: 26946082

32. Wassie L, Demissie A, Aseffa A, Abebe M, Yamuah L, Tilahun H, et al. Ex vivo cytokine mRNA levels correlate with changing clinical status of ethiopian TB patients and their contacts over time. Ratner A, editor. PLoS One. 2008; 3: e1522. https://doi.org/10.1371/journal.pone.0001522 PMID: 18231607

33. La Manna MP, Orlando V, Li Donni P, Sireci G, Di Carlo P, Cascio A, et al. Identification of plasma biomarkers for discrimination between tuberculosis infection/disease and pulmonary non tuberculosis disease. Ivanyi J, editor. PLoS One. 2018; 13: e0192664. https://doi.org/10.1371/journal.pone.0192664 PMID: 29543810

34. Vanham G, Toossi Z, Hirsch CS, Wallis RS, Schwander SK, Rich EA, et al. Examining a paradox in the pathogenesis of human pulmonary tuberculosis: immune activation and suppression/anergy. Tuber Lung Dis. 1997; 78: 145–58. Available: http://www.ncbi.nlm.nih.gov/pubmed/9713647 https://doi.org/10.1016/s0962-8479(97)90021-6 PMID: 9713647

35. Nie CQ, Bernard NJ, Norman MU, Amante FH, Lundie RJ, Crabb BS, et al. IP-10-mediated T cell homing promotes cerebral inflammation over splenic immunity to malaria infection. Riley EM, editor. PLoS Pathog. 2009; 5: e1000369. https://doi.org/10.1371/journal.ppat.1000369 PMID: 19343215

36. Druszczynska M, Kowalewicz-Kulbat M, Maszewska A, Rudnicka K, Szpakowski P, Wawrocki S, et al. The balance between pro- and anti-inflammatory cytokines in the immune responses to BCG and DTwP vaccines. Acta Biochim Pol. 2015; 62. https://doi.org/10.18388/abp.2015_1160 PMID: 26641637

37. Szpakowski P, Biet F, Locht C, Paszkiewicz M, Rudnicka W, Druszczyńska M, et al. Dendritic cell activity driven by recombinant *Mycobacterium bovis* BCG producing human il-18, in healthy BCG vaccinated adults. J Immunol Res. Hindawi Limited; 2015; 2015: 359153. https://doi.org/10.1155/2015/359153 PMID: 26339658

38. Chegou NN, Black GF, Loxton AG, Stanley K, Essone PN, Klein MR, et al. Potential of novel *Mycobacterium tuberculosis* infection phase-dependent antigens in the diagnosis of TB disease in a high burden setting. BMC Infect Dis. BioMed Central; 2012; 12: 10. https://doi.org/10.1186/1471-2334-12-10 PMID: 22260319

39. Braian C, Svensson M, Brighenti S, Lerm M, Parasa VR. A 3D human lung tissue model for functional studies on *Mycobacterium tuberculosis* infection; Infection. J Vis Exp. 2015; https://doi.org/10.3791/53084 PMID: 26485646

*pathogens*

MDPI

*Article*

# Interleukin-18, Functional IL-18 Receptor and IL-18 Binding Protein Expression in Active and Latent Tuberculosis

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**Abstract:** A thorough understanding of the processes modulating the innate and acquired immune response to *Mycobacterium tuberculosis* (*M.tb*) infection in the context of gene expression is still a scientific and diagnostic problem. The study was aimed to assess IL-18, IL-18 binding protein (IL-18BP), IL-18R, IFN-γ, and IL-37 mRNA expression in patients with active tuberculosis (ATB) and healthy volunteers with latent *M.tb*-infection (LTB) or *M.tb*-uninfected healthy controls (Control). The relative mRNA expression was assessed in the buffy coat blood fraction using the qPCR method. In total, 97 BCG-vaccinated Polish adults were enrolled in the study. The relative expression of IL-18 and IL-18BP mRNA was significantly elevated in the ATB and LTB groups. In ATB, but not LTB individuals, the overexpression of IL-18 and IL-18BP, as well as a significant increase in IFN-γ mRNA expression, might be considered as a manifestation of active tuberculosis disease. No statistically significant differences were observed in the IL-37 mRNA expression among the studied groups. Particularly noteworthy is the outstanding reduction in the relative expression of IL-18R mRNA in the LTB group as compared to the ATB and Control group. Reduced expression of IL-18R in LTB group may, at least partially, prevent the development of a pathological inflammatory reaction and promote the maintenance of homeostatic conditions between host immunity and *M.tb.*

**Keywords:** tuberculosis; IL-18; IL-18BP; IL-18R; gene expression

## 1. Introduction

Tuberculosis (TB) is the leading global cause of death from an infectious agent, *Mycobacterium tuberculosis* (*M.tb*). TB affects about 10-million people in the world and is a cause of two-million deaths annually, according to the estimates of the World Health Organization [1]. One-third of the world population carries an asymptomatic *M.tb* infection. These individuals have developed an efficient immune response that allows them to block the metabolic activity of the pathogen, but it does not provide its eradication. People with a latent TB infection (LTB) represent a reservoir of potential progress to disease, because about 5–10% of them will develop active TB disease, if not treated.

The antigen-specific, as well as non-specific, response of the immune cells to *M.tb* infection is modulated by mRNA expression, which results in the production of cytokines and other proteins activating numerous cell populations. Among these, interleukin (IL)-18plays an important

role—it induces NK cell cytotoxic activity and promotes the development of Th1 cell response. This mechanism is associated with the production of interferon (IFN)-γ, which is a key element in anti-mycobacterial protection. IL-18 was first described in 1989 as an "IFN-γ inducing factor" [2–4]. Similarly to IL-1β, IL-18 is constantly synthesized as an inactive precursor, and the cysteine protease (caspase-1) is involved in its maturation. In a mouse model, increased susceptibility to infection with *M.tb* was found in animals that were not able to produce IL-18 [5]. Moreover, IL-18 increases the expression of adhesion molecules, the synthesis of enzyme nitric oxide synthase, and the production of chemokines. In addition to inducing a T helper (h) type 1 (Th1) cellular response, IL-18, together with IL-2, leads to a Th2 type cell response and the production of IL-4 and IL-13 [6]. In humans, the gene encoding IL-18 is located on chromosome 11 at position 11q22.2–q22.3 and it consists of six exons. There are several common polymorphisms in the promoter region of IL-18 than affect the transcription factor binding sites and, in turn, might be expression quantitative trait loci (eQTLs) for IL-18. Therefore, these genetic variants may predispose to TB by affecting the expression of the cytokine itself, followed by the development of the IFN-γ-mediated Th1 response [7].

The IL-18 levels are also regulated by soluble IL-18 binding protein (IL-18BP), which is a natural inhibitor of IL-18. Under physiological conditions, the concentration of plasma IL-18BP is ~20 times higher than that of IL-18, which prevents IL-18 from binding to its cellular receptor. The gene coding for IL-18BP is located on chromosome 11 at position 11q13.4. The mRNA promoter region contains two response elements (RE), regulatory sequences sensitive to IFN-γ attachment, which results in increased gene expression and protein production [8,9].

Cell activation by IL-18 occurs via the IL-18R receptor, which belongs to the IL-1 family, the members of which show structural and functional similarity. IL-18R is expressed on many cells, such as macrophages, NK cells, neutrophils, epithelial cells, and smooth muscle. The IL-18R receptor is a heterodimer that is composed of two polypeptide chains: IL-18Rα and IL-18Rβ. The IL-18Rα chain is responsible for ligand binding. However, it binds to IL-18 with low affinity. On the other hand, the IL-18Rβ chain functions as a co-receptor, which enhances the strength with which the receptor binds IL-18 and transmits the signal to the inside of the cell [10–12]. The genes encoding the receptor are located on chromosome 2 at position 2q12.1 There are known genetic variants in the regulatory regions of IL-18R associated genes, which may affect its and functionality [13].

A new member of the IL-1 family, IL-37, has gained increasing attention in recent years. IL-37, to which IL-18BP also has a high affinity, was found to be an important regulator of inflammation [14,15]. IL-37 is also able to bind to IL-18Rα, but with much lower affinity than IL-18 [16]. IL-18BP and IL-37 act to reduce the production of inflammatory cytokines; however, the anti-inflammatory properties of IL-37 depend on the concentration of IL-18 binding protein. The human IL-37 gene cluster is located on chromosome 2 at position 2q14.1 [17].

IFN-γ is a cytokine that is known to be crucial in regulating the immune response to *M.tb* infection. This cytokine is mainly produced by activated CD4 (+) Th1 T lymphocytes, and it also has a key role in inducing nitric oxide (NO)-dependent apoptosis of mycobacteria infected macrophages. IFN-γ was shown to increase the expression of major histocompatibility complex (MHC) class I and II surface molecules and promote differentiation towards Th1 response. Deleterious mutations in the gene encoding the IFN-γ receptor predispose to the acute course of mycobacterial infection [3,18]. The human immune interferon gene is located on chromosome 12at position 12q15 [19]. The expression levels of these cytokines, as well as their mutual relations in sera, as well as in cultures stimulated, were found to be informative of *M.tb* infection status [20,21]. This work aims to assess the level of expression of IL-18, IL-18BP, IL-18R, as well as IFN-γand IL-37 genes in patients with active pulmonary tuberculosis (ATB), healthy individuals with latent *M.tb* infection (LTB), and healthy uninfected controls (Control).

## 2. Materials and Methods

### *2.1. Study Subjects*

A study group consisted of 97 adults that were vaccinated with *M. bovis* BCG in childhood, including 51 patients with active pulmonary ATB (40 males, 11 females) aged 23–80 years, hospitalized and diagnosed in the Regional Center Hospital for Tuberculosis, Lung Diseases, and Rehabilitation in Lodz, Poland, 24 healthy volunteers, eight males, 16 females, with LTB (25–65 years old), and 22 healthy uninfected Controls (five males, 17 females), aged 18–66 years (Table 1). Active pulmonary TB was diagnosed by chest radiography and standard clinical examination—by Ziehl–Neelsen staining of sputum smears and *M.tb* culture as a gold standard. The Ethics Committee of the University in Lodz, Poland approved the study (ethical approval number 17/KBBN-UŁ/II/2016; date 2016/11/10). Informed consent to use blood for research purposes was signed by all participants.

**Table 1.** Patient characteristics.

| | ATB | LTB | Control |
|---|---|---|---|
| N | 51 | 24 | 22 |
| Sex M/F | 40/11 * | 8/16 | 5/17 * |
| Ethnicity | Caucasian | Caucasian | Caucasian |
| Age | | | |
| median | 54 | 51 | 37 |
| range | 23–80 | 25–65 | 18–66 |
| years (IQR) | 42–63 | 45–57 | 27–42 |
| BCG vaccination | 100% | 100% | 100% |
| QFT result, N (%) | | | |
| positive | 22 (43%) | 24 (100%) | 0 (0%) |
| negative | 28 (57%) | 0 (0%) | 22 (100%) |

Abbreviations: ATB—active tuberculosis patients; LTB—latently *M.tb* infected individuals; Control—*M.tb*-uninfected healthy controls; QFT—QuantiFERON TB Gold test. * The proportion of men in the ATB group was significantly higher than in the Control group ($p < 0.05$).

### *2.2. RNA Isolation*

RNA isolation from the buffy coat obtained after centrifugation (150 g, 4 °C, 10 min.) was performed by the use of a commercial QIAamp® RNA Blood Mini set. Genetic material was isolated from 3.5 mL of peripheral blood obtained from all volunteers using EDTA tubes and the BD Vacutainer® Blood Collection system. The isolation process was fully compliant with the manufacturer's guidelines. The isolation process was extended by an additional purification step using the RNase-Free DNase Set to obtain the purest product free of any genomic DNA. All of the procedures were carried out within no more than 2 h from the collection of a blood sample. Part of extracted RNA was used to visualize a product and obtain cDNA immediately after the isolation process; the rest of the genetic material was stored at −80 °C until analyzed.

### *2.3. Spectrophotometric Evaluation of Isolated RNA and Gel Visualization*

At the end of the isolation procedure, 1.5 µL of each sample was pipetted into a sterile Eppendorf tube, which was then placed in an ice block, in order to assess the quality and quantity of RNA obtained. An additional water-containing blank was prepared to calibrate the device (NanoDrop), which was used for RNA elution in the final isolation step. For visualization of RNA, 1.2% agarose gel was prepared based on TAE buffer. Agarose gel was enriched after cooling with 10 µL of 5 mg/mL ethidium bromide. Each sample of RNA in a volume of 3.5 µL was heated to 70 °C in a water bath for 1 min. and then cooled on ice for another minute. An equal amount of loading buffer was then added to each of the samples, and the mixture was then loaded to the gel. The GeneRuler Plus DNA Ladder

size 100 bp from ThermoFisher was used as the size standard. Electrophoresis was carried out at 90 V for 60 min. Subsequently, to visualize the obtained product, the gel was transferred to a Gel-Doc 2000 apparatus that was connected to a computer with Quantity One software. The analysis of the obtained gel allowed a clear distinction between the two isolated RNA fractions: 18S RNA and 28S RNA.

### *2.4. Reverse Transcription*

cDNA was synthesized according to the manufacturer's instructions of the iScript™ cDNA Synthesis Kit (Bio-Rad). In the first stage of the reverse transcription reaction, 1 µg matrix RNA previously tested for quality and integrity was transferred from an isolated sample to 0.2 µL Eppendorf tubes, followed by the addition of reagents that are necessary for the cDNA synthesis process according to the manufacturer's proportions, which results in a mixture with a final volume of 20 µL. The reverse transcription reaction was carried out in a Biometra UNO II thermal cycler under conditions following the manufacturer's guidelines. The resulting cDNA was stored at −20 °C until analyzed.

### *2.5. qPCR Reaction*

qPCR was performed in a CFX96 Real-Time PCR Detection System (Bio-Rad). The reaction mixture (10 µL) contained 5 µL of iTaq universal SYBR Green Supermix, 0.5 µL of each primer (10 µM), 1 µL of cDNA, and 3 µL of nuclease-free water. Amplifications were performed using the following cycling profile: an initial activation step (95 °C for 3 min.) followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at a temperature appropriate for selected starters (Table 2) for 10 s, and extension at 72 °C for 20 s. For melting curve analysis, a dissociation step cycle (60 °C for 5 s, and then 0.5 °C for 5 s until 95 °C) was added. All of the qRT-PCR experiments were performed in three technical replicas.

**Table 2.** Starters and temperatures of annealing selected for expression analysis.

| | Sequence | Temperature of Annealing | Source |
|---|---|---|---|
| IL-18 | forward 5′-GCTTGAATCTAAATTATCAGTC-3′<br>reverse 5′-GAAGATTCAAATTGCATCTTAT-3′ | 55 °C | [22] |
| IL-18BP | forward 5′-CAACTGGACACCAGACCTCA-3′<br>reverse 5′-AGCTCAGCGTTCCATTCAGT-3′ | 64 °C | [23] |
| IL-18R | forward 5′-GGACTCCATGAAGCATTGGT-3′<br>reverse 5′-AGACTCGGAAAGAACAGGCA-3′ | 58 °C | [24] |
| IFN-γ | forward 5′-CTCTTGGCTGTTACTGCCAGG-3′<br>reverse 5′-CTCCACACTCTTTTGGATGCT-3′ | 60 °C | [25] |
| IL-37 | Sino Biological INC. | 60 °C | - |

Analysis of gene expression was done through a comparative method (ΔΔCt) in order to determine the relative level of expression of selected mRNAs. This method is based on calculating the differences in the level of expression of the test gene and the reference gene. The calculations use the threshold cycle (Ct) values of the qPCR reaction. Ct values were determined for both the test and reference genes in both the test and control samples, for which the differences between the individual Ct values (ΔCt) were then calculated.

### *2.6. Statistical Analysis*

The expression of genes between the study groups was compared using Kruskal–Wallis' non-parametric diagnostic test. A $p$-value $< 0.05$ was considered to be statistically significant. Statistical analyses were done using MedCalc (MedCalc Software, Ostend, Belgium) and GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) software.

## 3. Results

### *3.1. Selection of Reference Genes*

We had chosen to use the Reference Gene qPCR Panel BioRad with primers being designed and coated on the plate in lyophilized form for selected reference genes adequate for the analyzed material obtained from volunteers [26]. The housekeeping genes were: ACTB, RPL13A, B2M, RPLP0, G6PD, RPS18, GAPDH, TBP, GUSB, TFRC, HMBS, YWHAZ, HPRT1, PGK1, and IPO8. Additionally, the panel contained several internal controls ensuring the most reliable results. After the RT-PCR reaction, the analysis of the melting curves for the obtained amplicons was carried out in order to determine the quality of the reaction. In the next stage, the Ct values that were detected for individual genes were used for further analyses, which were carried out using the BioRad program (CFX Manager™ Software), and the GeNorm program. As a consequence of the analysis, we decided to use the following reference genes: GADPH, HPRT1, and TBP. These genes had the lowest M value corresponding to the most stable gene expression in the sample tested. The analysis of Ct values using the geNorm program allowed for further refinement of this gene list and, in turn, only HRTP1 and GAPDH were used as the reference panel, as shown in Figure 1.

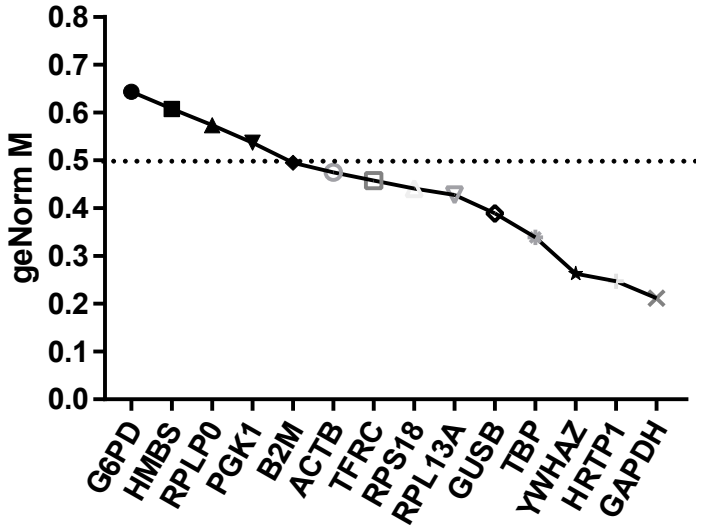


**Figure 1.** Chart showing the most (lowest M value) and least stable (highest M value) reference genes indicated by the program GeNorm.

Melt peak analysis demonstrated a single homogenous peak for all primer sets, including selected reference genes (Figure 2).

### *3.2. IL-18, IL-18BP, IL-18R, IFN-γ, and IL-37 Gene Expression in the Studied Groups*

A significantly higher relative level of IL-18 mRNA expression was observed in LTB individuals as compared to healthy controls without *M.tb* infection ($p = 0.023$). A similar increase in the relative IL-18 expression level was observed among ATB patients; however, the difference in values for the group ATB and group Control group was not significant ($p = 0.082$) (Figure 3A).

The relative level of IL-18BP mRNA expression was significantly higher in ATB patients ($p < 0.001$) and healthy LTB individuals ($p = 0.006$) than in Control group volunteers (Figure 3B).

There were major significant differences between the three groups in the IL-18R mRNA expression. In the LTB groups, the relative level of IL-18R mRNA was much lower than in the ATB patients ($p < 0.001$) and individuals from the Control group ($p < 0.001$) (Figure 3C).

The level of relative expression of IFN-γ mRNA was significantly higher in active TB patients ($p = 0.002$) and LTB individuals ($p = 0.029$) than in the Control group (Figure 3D).

No statistically significant differences were observed in the levels of relative expression of IL-37 mRNA among the studied groups (Figure 3E).

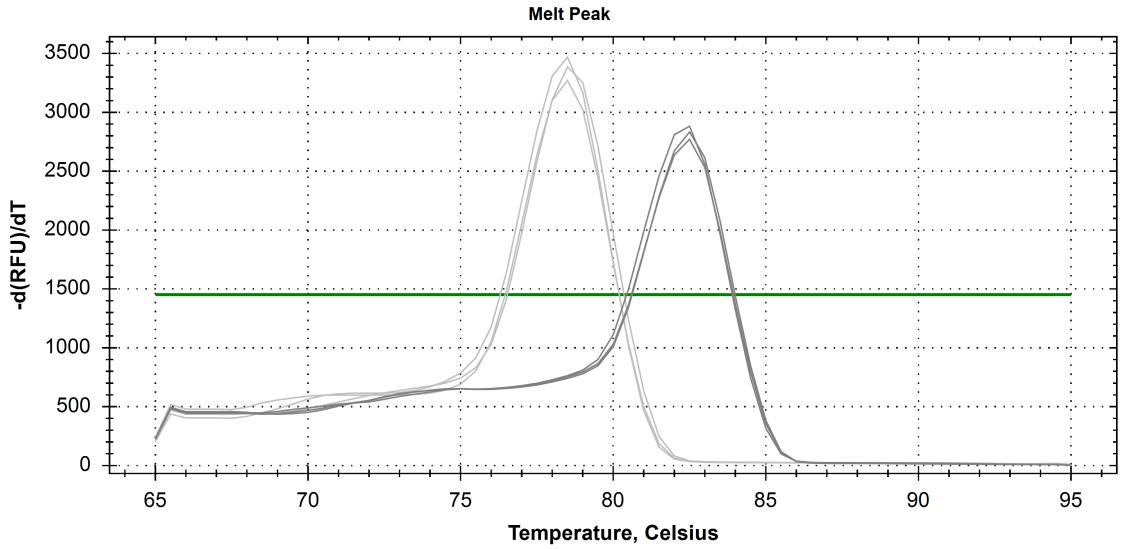


**Figure 2.** Melt peak analysis of reference genes HPRT1 (light grey) and GAPDH (dark grey).

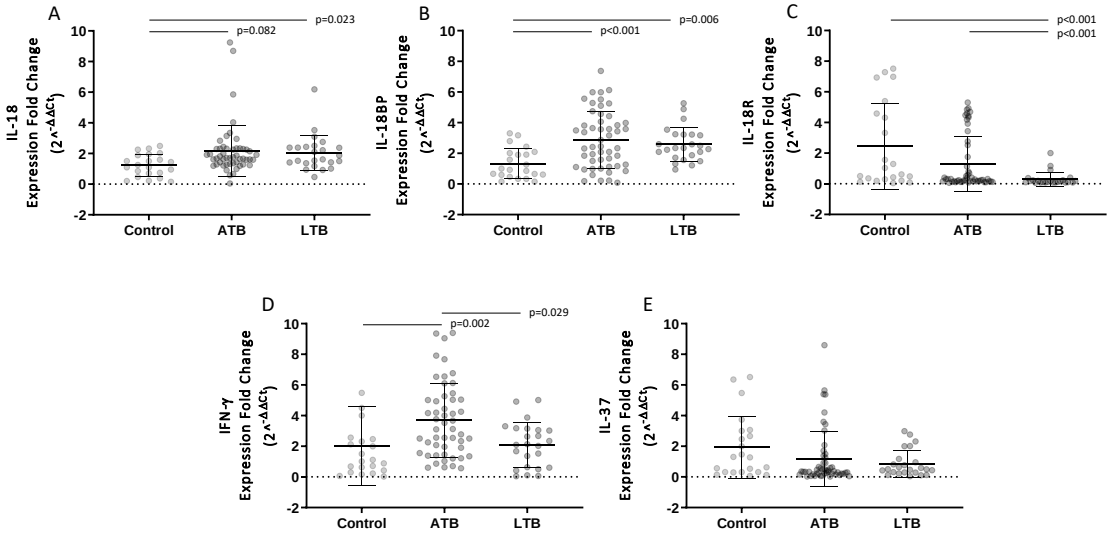


**Figure 3.** Relative expression of IL-18, IL-18BP, IL-18R, IFN-γ, and IL-37 mRNA in studied groups. Dot plot with mean (horizontal line), and standard deviation (whiskers), IL-18 (**A**), IL-18BP (**B**), IL-18R (**C**), IFN-γ (**D**), IL-37 (**E**) in the groups of healthy volunteers (Control), patients with active tuberculosis (ATB), and latently infected individuals (LTB).

## 4. Discussion

A unique feature of *M.tb* is the ability to persist in the host for a long time, despite functioning mechanisms of acquired immunity. In turn, approximately 2.3 billion individuals have latent tuberculosis infection without evidence of the clinical manifestation of active TB. It is hypothesized that active TB is usually caused by the reactivation of endogenous infection and untreated LTB is a

major source of new active TB infections and transmission. The risk for severe active tuberculosis reactivation is increased several times among the immunocompromised individuals, diabetics, organ transplantation recipients, patients with hematologic malignancies, or HIV-infected subjects. Several treatment regimens of LTB are recommended, between isoniazid monotherapy for six months, rifampicin plus isoniazid for three months or rifapentine plus isoniazid for three months [27]. Currently, interferon-gamma release assays (IGRA) are used to test LTB based on the production of IFN-γ by Th1 cells responding to specific *M.tb* antigens, although the IGRA's accuracy in immunocompromised individuals is still limited. Moreover, the definition of active tuberculosis infection might not be accurate using IFN-γ responses to *M.tb* antigens even in combination with tuberculin skin test [28]. At the same time, neither the IGRA test nor the tuberculin skin test can distinguish between latent and active TB infection. Strategies for rapid differentiation of patients with active TB and people with LTB and prevention of tuberculosis reactivation in LTB individuals are urgently needed.

To meet such needs, we compared the expression of genes of the IL-18 pathway, functional receptor of this cytokine IL-18R, and IFN-γ, as well as the expression of IL-18BP and IL-37 genes in groups of patients with active TB, healthy individuals with LTB and healthy controls without *M.tb* infection.

Our results for the first time showed a significant increase in the relative expression of IL-18 and IL-18BP mRNA in the group of patients with active TB and LTB individuals when compared to healthy controls, according to available literature data. It might suggest a permanent activation of the immune cell signaling pathways in the course of *M.tb* infection either in the control or progression to active TB disease. Moreover, no significant differences in relative IL-18 and IL-18BP mRNA expression were observed between active TB patients and LTB individuals. Pechkovsky et al. demonstrated an increased expression of IL-18 mRNA in type II lung epithelial cells obtained from patients with pulmonary TB. Pneumocytes that were cultured in the presence of *M.tb* cell lysate showed an increased IL-18 mRNA expression in comparison with unstimulated cells and pneumocytes stimulated with PPD or LPS [29]. Higher levels of IL-18 mRNA expression were observed in monocytes responding to *M. leprae* antigens [30]. Corbaz et al. showed significantly higher levels of IL-18 and IL-18BP mRNA expression in the intestinal mucosa of patients with Crohn's disease as compared to healthy controls. We detected a similar increase in IL-18 and IL-18BP mRNA expression in the group of patients with active TB and healthy LTB individuals. These results are somewhat out of line with our earlier definition of a simultaneous increase in serum IL-18 and IL-18BP protein expression, which might be treated as a discriminatory biomarker of active tuberculosis and LTB. Yet, it is worth noting that only the complex co-expression of serum IL-18BP and IL-37, IP-10, and IFN-γ were identified as the most accurate discriminative biomarker set for diagnosis of active TB [21].

Our study allowed for the discovery of a novel relation—the significantly lower expression of functional IL-18R receptor mRNA in the LTBI group as compared to the active TB and healthy controls. This low expression of IL-18R mRNA in LTB individuals was accompanied by IFN-γ mRNA expression at the 'baseline' level, characterizing healthy individuals without *M.tb* infection. Similarly, Taha et al. pointed out that the expression of IFN-γ genes was significantly higher in the group of patients with active TB as compared to those who were infected but did not develop active TB [31].

Among many possible mediators of host response to *M.tb* is the activity of the indoleamine 2,3-dioxygenase-1 (IDO1), the enzyme of tryptophan metabolism, which leads to the formation of tryptophan metabolites, including quinolinic and picolinic acids [32]. In animal models, increased IDO-1 expression and the activation of the tryptophan-kynurenine pathway were indicated to play a crucial role in *M.tb* pathogenesis [29,30,33,34]. The depletion of tryptophan, which is required for microbial growth, as well as the accumulation of biologically active tryptophan metabolites, impaired effective anti-mycobacterial immune response and, thus, favoured survival and persistence of the pathogen [32]. In *M.tb*-infected mice, IFN-γ receptor-deficiency in nonhaematopoietic cells led to a lack of IDO-1 expression and it was associated with exuberant neutrophil recruitment and increased mortality [33]. In macaques, the suppression of IDO activity led to the reduction of the bacterial burden and clinical symptoms of active TB that was accompanied by increased lung T cell proliferation, the induction of

inducible bronchus-associated lymphoid tissue, and the relocation of effector T cells to the center of the granuloma [34]. Mehra et al. demonstrated that IDO induction in the periphery of the granuloma correlated with active TB disease [30]. A few studies have investigated IDO-mediated tryptophan metabolism and its metabolites in humans in the context TB. Li et al. demonstrated increased IDO expression and activity in the pleural fluid from TB patients [35]. Almeida et al. found significantly higher expression levels of immune-suppressive mediators, including IDO-1, in patients with active pulmonary TB as compared to patients with other infectious lung diseases and healthy volunteers [36]. The authors suggested that the increased levels of immunosuppressive mediators may render the immune activation and counteract the development of Th1-type immune response against *M.tb*. The IDO levels were elevated at the time of TB diagnosis and declined after TB treatment, which serves as evidence that IDO expression might be both: a useful diagnostic marker of active TB as well as prognostic factor in TB treatment of HIV-negative patients [36]. Additionally, Adu-Gyamfi et al. showed that plasma IDO expression is a potential biomarker of active TB in HIV-positive patients [37], while Shi et al. confirmed that IDO activity might have an auxiliary diagnosis value for the early discrimination of multi-drug resistant TB patients [38]. The increase in IDO activity was noticed in both HIV-infected and uninfected active TB patients as compared with individuals with latent TB infection [36,37,39]. In relation to this statement, the simultaneous reduction of IL-18R mRNA expression together with significant overexpression of IL-18 mRNA observed by us in the LTB group is of particular interest.

The presented results entitle us to hypothesize that: the increase in IL-18 gene expression, the lack of increase in IFN-γ gene expression, and the remarkably reduced expression of IL-18R gene may be a novel set of conditions that partially describe the homeostasis between *M.tb* and host-immunity in latent tuberculosis infection. As an obligate intracellular pathogen, *M.tb* has numerous adaptive mechanisms of modifying cellular processes in the fight against the host immune response. In latent TB infection, *M.tb* bacilli benefit from epigenetic changes that occurred in the host immune system under mycobacterial infection [40]. These changes make the *M.tb* favorable environment in the host cells and promote mycobacterial survival, growth, and latency. In a study that was conducted among Chinese patients with pulmonary TB and healthy controls, single nucleotide polymorphisms in the IL-18R promoter were associated with genotype-specific methylation status and genotype-specific IL-18R expression [41]. In the author's opinion, the relationship between decreased mRNA expression of IL-18R that is caused by an SNP and increased DNA methylation can partially mediate the susceptibility to TB risk. No statistically significant differences were observed in the relative mRNA IL-37 expression among the groups in our study. IL-37 is a new member of the IL-1 family, which reduces systemic and local inflammation. IL-37 is expressed in various cells and tissues and it is regulated by numerous inflammatory stimuli and cytokines via different signal transduction pathways [42]. Mannose-capped lipoarabinomannan purified from *M.tb* induces IL-37 production via enhancing TLR2 expression in human type II alveolar epithelial cells; this process might contribute to the persistence of *M.tb* infection [43]. Zhao et al. indicated that IL-37 is also a negative regulator of immune responses in *Listeria monocytogenes* infection due to reduced production of colony-stimulating factors and increased macrophage apoptosis [44]. In our earlier studies, the serum concentration of the IL-37 protein was similar in the group of patients with active pulmonary TB and healthy individuals with or without latent *M.tb* infection [21]. However, the complex co-expression between the two IL-18 inhibitors, IL-18BP and IL-37, was identified as the strongest discriminative biomarker of active TB disease.

## 5. Conclusions

The role of IL-18, its binding protein IL-18BP, and IFN-γ in the development of the immune response against mycobacteria was confirmed by observing the increased level of the expression of these genes in the group of patients with active pulmonary TB. The reduced expression of IL-18R gene in healthy individuals with latent TB infection can, at least partially, prevent the development of a pathological inflammatory reaction and promote the maintenance of homeostatic conditions between

host immunity and *M.tb* infection. In our future studies, we plan to test the expression of other genes that are tightly co-regulated with the IL-18 pathway.

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## References

1. World Health Organisation. *Global Tuberculosis Report 2018*; World Health Organization: Geneva, Switzerland, 2018. Available online: https://apps.who.int/iris/handle/10665/274453 (accessed on 5 June 2020).
2. Ahmad, S. Pathogenesis, immunology, and diagnosis of latent mycobacterium tuberculosis infection. *Clin. Dev. Immunol.* **2011**, *2011*, 814943. [CrossRef] [PubMed]
3. Akdis, M.; Aab, A.; Altunbulakli, C.; Azkur, K.; Costa, R.A.; Crameri, R.; Duan, S.; Eiwegger, T.; Eljaszewicz, A.; Ferstl, R.; et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* **2016**, *138*, 984–1010. [CrossRef] [PubMed]
4. Wawrocki, S.; Druszczynska, M.; Kowalewicz-Kulbat, M.; Rudnicka, W. Interleukin 18 (IL-18) as a target for immune intervention. *Acta Biochim. Pol.* **2016**, *63*, 59–63. [CrossRef] [PubMed]
5. Schneider, B.E.; Korbel, D.; Hagens, K.; Koch, M.; Raupach, B.; Enders, J.; Kaufmann, S.H.E.; Mittrücker, H.-W.; Schaible, U.E. A role for IL-18 in protective immunity against *Mycobacterium tuberculosis*. *Eur. J. Immunol.* **2010**, *40*, 396–405. [CrossRef] [PubMed]
6. Nakanishi, K.; Yoshimoto, T.; Tsutsui, H.; Okamura, H. Interleukin-18 regulates both Th1 and Th2 response. *Annu. Rev. Immunol.* **2001**, *19*, 423–474. [CrossRef] [PubMed]
7. Wawrocki, S.; Kielnierowski, G.; Rudnicka, W.; Druszczynska, M. Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population. *Acta Biochim. Pol.* **2019**, *66*, 337–342. [CrossRef] [PubMed]
8. Novick, D.; Kim, S.; Kaplanski, G.; Dinarello, C.A. Interleukin-18, more than a Th1 cytokine. *Semin. Immunol.* **2013**, *25*, 439–448. [CrossRef]
9. Dinarello, C.A.; Novick, D.; Kim, S.; Kaplanski, G. Interleukin-18 and IL-18 binding protein. *Front. Immunol.* **2013**, *4*, 1–10. [CrossRef]
10. Gracie, J.A.; Robertson, S.E.; Mcinnes, I.B. Interleukin-18 Abstract. *J. Leukoc. Biol.* **2003**, *73*, 213–224. [CrossRef]
11. Huang, Y.; Li, D.; Zhang, P.; Liu, M.; Liang, X.; Yang, X.; Jiang, L.; Zhang, L.; Zhou, W.; Su, J.; et al. IL-18R-dependent and independent pathways account for IL-18-enhanced antitumor ability of CAR-T cells. *FASEB J.* **2020**, *34*, 1768–1782. [CrossRef]
12. Gutzmer, R.; Langer, K.; Mommert, S.; Wittmann, M.; Kapp, A.; Werfel, T. Human Dendritic Cells Express the IL-18R and Are Chemoattracted to IL-18. *J. Immunol.* **2003**, *171*, 6363–6371. [CrossRef] [PubMed]
13. Tiret, L.; Godefroy, T.; Lubos, E.; Nicaud, V.; Tregouet, D.-A.; Barbaux, S.; Schnabel, R.; Bickel, C.; Espinola-Klein, C.; Poirier, O.; et al. Genetic analysis of the interleukin-18 system highlights the role of the interleukin-18 gene in cardiovascular disease. *Circulation* **2005**, *112*, 643–650. [CrossRef] [PubMed]
14. Nold, M.F.; Nold-Petry, C.A.; Zepp, J.A.; Palmer, B.E.; Bufler, P.; Dinarello, C.A. IL-37 is a fundamental inhibitor of innate immunity. *Nat. Immunol.* **2010**, *11*, 1014–1022. [CrossRef] [PubMed]
15. Dinarello, C.A.; Nold-Petry, C.; Nold, M.; Fujita, M.; Li, S.; Kim, S.; Bufler, P. Suppression of innate inflammation and immunity by interleukin-37. *Eur. J. Immunol.* **2016**, *46*, 1067–1081. [CrossRef]
16. Quirk, S.; Agrawal, D.K. Immunobiology of IL-37: Mechanism of action and clinical perspectives. *Expert Rev. Clin. Immunol.* **2014**, *10*, 1703–1709. [CrossRef]
17. Pan, Y.; Wen, X.; Hao, D.; Wang, Y.; Wang, L.; He, G.; Jiang, X. The role of IL-37 in skin and connective tissue diseases. *Biomed. Pharmacother.* **2020**, *122*, 109705. [CrossRef]

18. Donovan, M.L.; Schultz, T.E.; Duke, T.J.; Blumenthal, A. Type I interferons in the pathogenesis of tuberculosis: Molecular drivers and immunological consequences. *Front. Immunol.* **2017**, *8*, 1633. [CrossRef]
19. Naylor, S.L.; Sakaguchi, A.Y.; Shows, T.B.; Law, M.L.; Goeddel, D.V.; Gray, P.W. Human immune interferon gene is located on chromosome 12. *J. Exp. Med.* **1983**, *157*, 1020–1027. [CrossRef]
20. Druszczynska, M.; Wlodarczyk, M.; Kielnierowski, G.; Seweryn, M.; Wawrocki, S.; Rudnicka, W. CD14-159C/T polymorphism in the development of delayed skin hypersensitivity to tuberculin. *PLoS ONE* **2017**, *12*, e0190106. [CrossRef]
21. Wawrocki, S.; Seweryn, M.; Kielnierowski, G.; Rudnicka, W.; Wlodarczyk, M.; Druszczynska, M. IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB. *PLoS ONE* **2019**, *14*, e0225556. [CrossRef]
22. Wang, Y.; Shan, N.; Chen, O.; Gao, Y.; Zou, X.; Wei, D.; Wang, C.; Zhang, Y. Imbalance of Interleukin-18 and Interleukin-18 Binding Protein in Children with Henoch-Schönlein Purpura. *J. Int. Med. Res.* **2011**, *39*, 2201–2208. [CrossRef] [PubMed]
23. Reale, M.; Kamal, M.A.; Patruno, A.; Costantini, E.; D'Angelo, C.; Pesce, M.; Greig, N.H. Neuronal cellular responses to extremely low frequency electromagnetic field exposure: Implications regarding oxidative stress and neurodegeneration. *PLoS ONE* **2014**, *9*, 1–14. [CrossRef] [PubMed]
24. Yoshino, O.; Osuga, Y.; Koga, K.; Tsutsumi, O.; Yano, T.; Fujii, T.; Kugu, K.; Momoeda, M.; Fujiwara, T.; Tomita, K.; et al. Evidence for the expression of interleukin (IL)-18, IL-18 receptor and IL-18 binding protein in the human endometrium. *MHR Basic Sci. Reprod. Med.* **2001**, *7*, 649–654. [CrossRef] [PubMed]
25. Zumwalt, T.J.; Arnold, M.; Goel, A.; Boland, C.R. Active secretion of CXCL10 and CCL5 from colorectal cancer microenvironments associates with GranzymeB+ CD8+ T-cell infiltration. *Oncotarget* **2015**, *6*, 2981–2991. [CrossRef]
26. Bustin, S.A.; Benes, V.; Garson, J.A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M.W.; Shipley, G.L.; et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **2009**, *55*, 611–622. [CrossRef]
27. World Health Organisation. *Latent Tuberculosis Infection Updated and Consolidated Guidelines for Programmatic Management*; World Health Organization: Geneva, Switzerland, 2018. Available online: https://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/ (accessed on 5 June 2020).
28. Wlodarczyk, M.; Rudnicka, W.; Janiszewska-Drobinska, B.; Kielnierowski, G.; Kowalewicz-Kulbat, M.; Fol, M.; Druszczynska, M. Interferon-gamma assay in combination with tuberculin skin test are insufficient for the diagnosis of culture-negative pulmonary tuberculosis. *PLoS ONE* **2014**, *9*, 1–12. [CrossRef]
29. Moreau, M.; Lestage, J.; Verrier, D.; Mormède, C.; Kelley, K.W.; Dantzer, R.; Castanon, N. Bacille Calmette-Guérin inoculation induces chronic activation of peripheral and brain indoleamine 2,3-dioxygenase in mice. *J. Infect. Dis.* **2005**, *192*, 537–544. [CrossRef]
30. Mehra, S.; Alvarez, X.; Didier, P.J.; Doyle, L.A.; Blanchard, J.L.; Lackner, A.A.; Kaushal, D. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by Mycobacterium tuberculosis. *J. Infect. Dis.* **2013**, *207*, 1115–1127. [CrossRef]
31. Taha, R.A.; Kotsimbos, T.C.; Song, Y.L.; Menzies, D.; Hamid, Q. IFN-γ and IL-12 are increased in active compared with inactive tuberculosis. *Am. J. Respir. Crit. Care Med.* **1997**, *155*, 1135–1139. [CrossRef]
32. Blumenthal, A.; Nagalingam, G.; Huch, J.H.; Walker, L.; Guillemin, G.J.; Smythe, G.A.; Ehrt, S.; Britton, W.J.; Saunders, B.M.M. tuberculosis induces potent activation of IDO-1, but this is not essential for the immunological control of infection. *PLoS ONE* **2012**, *7*, 1–14. [CrossRef]
33. Desvignes, L.; Ernst, J.D. Interferon-γ-Responsive nonhematopoietic cells regulate the immune response to Mycobacterium tuberculosis. *Immunity* **2009**, *31*, 974–985. [CrossRef] [PubMed]
34. Gautam, U.S.; Foreman, T.W.; Bucsan, A.N.; Veatch, A.V.; Alvarez, X.; Adekambi, T.; Golden, N.A.; Gentry, K.M.; Doyle-Meyers, L.A.; Russell-Lodrigue, K.E.; et al. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of Mycobacterium tuberculosis. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E62–E71. [CrossRef] [PubMed]
35. Li, Q.; Li, L.; Liu, Y.; Fu, X.; Qiao, D.; Wang, H.; Lao, S.; Huang, F.; Wu, C. Pleural fluid from tuberculous pleurisy inhibits the functions of T cells and the differentiation of Th1 cells via immunosuppressive factors. *Cell. Mol. Immunol.* **2011**, *8*, 172–180. [CrossRef] [PubMed]

36. Almeida, A.S.; Lago, P.M.; Boechat, N.; Huard, R.C.; Lazzarini, L.C.O.; Santos, A.R.; Nociari, M.; Zhu, H.; Perez-Sweeney, B.M.; Bang, H.; et al. Tuberculosis is associated with a down-modulatory lung immune response that impairs th1-type immunity. *J. Immunol.* **2009**, *183*, 718–731. [CrossRef]
37. Adu-Gyamfi, C.G.; Snyman, T.; Hoffmann, C.J.; Martinson, N.A.; Chaisson, R.E.; George, J.A.; Suchard, M.S. Plasma indoleamine 2, 3-dioxygenase, a biomarker for tuberculosis in human immunodeficiency virus-infected patients. *Clin. Infect. Dis.* **2017**, *65*, 1356–1358. [CrossRef]
38. Shi, W.; Wu, J.; Tan, Q.; Hu, C.M.; Zhang, X.; Pan, H.Q.; Yang, Z.; He, M.Y.; Yu, M.; Zhang, B.; et al. Plasma indoleamine 2,3-dioxygenase activity as a potential biomarker for early diagnosis of multidrug-resistant tuberculosis in tuberculosis patients. *Infect. Drug Resist.* **2019**, *12*, 1265–1276. [CrossRef]
39. Suzuki, Y.; Suda, T.; Asada, K.; Miwa, S.; Suzuki, M.; Fujie, M.; Furuhashi, K.; Nakamura, Y.; Inui, N.; Shirai, T.; et al. Serum indoleamine 2,3-dioxygenase activity predicts prognosis of pulmonary tuberculosis. *Clin. Vaccine Immunol.* **2012**, *19*, 436–442. [CrossRef]
40. Yadav, V.; Dwivedi, V.P.; Bhattacharya, D.; Mittal, A.; Moodley, P.; Das, G. Genetics and genome research understanding the host epigenetics in Mycobacterium tuberculosis infection. *J. Genet Genome Res.* **2015**, *2*, 1. [CrossRef]
41. Zhang, J.; Zheng, L.; Zhu, D.; An, H.; Yang, Y.; Liang, Y.; Zhao, W.; Ding, W.; Wu, X. Polymorphisms in the interleukin 18 receptor 1 gene and tuberculosis susceptibility among Chinese. *PLoS ONE* **2014**, *9*, e110734. [CrossRef]
42. Wang, L.; Quan, Y.; Yue, Y.; Heng, X.; Che, F. Interleukin-37: A crucial cytokine with multiple roles in disease and potentially clinical therapy (Review). *Oncol. Lett.* **2018**, *15*, 4711–4719. [CrossRef]
43. Huang, Z.; Zhao, G.W.; Gao, C.H.; Chi, X.W.; Zeng, T.; Hu, Y.W.; Zheng, L.; Wang, Q. Mannose-capped lipoarabinomannan from mycobacterium tuberculosis induces IL-37 production via upregulating ERK1/2 and p38 in human type ii alveolar epithelial cells. *Int. J. Clin. Exp. Med.* **2015**, *8*, 7279–7287. [PubMed]
44. Zhao, M.; Hu, Y.; Shou, J.; Su, S.B.; Yang, J.; Yang, T. IL-37 impairs host resistance to Listeria infection by suppressing macrophage function. *Biochem. Biophys. Res. Commun.* **2017**, *485*, 563–568. [CrossRef] [PubMed]


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Original article

# IL-18 and related function proteins associated with tuberculosis severity and screening for active TB among patients with non-mycobacterial community-acquired pneumonia (CAP)

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ABSTRACT

*Background:* Differentiation of active pulmonary tuberculosis (TB) from non-mycobacterial community-acquired pneumonia (CAP) still remains a diagnostic challenge.
*Objective:* The study aimed to quantify the IL-18, IFN-γ, IL-18BP, IL-37, and IP-10 levels in serum and *Mycobacterium tuberculosis* (*M.tb*) antigens-stimulated blood cultures from TB or CAP patients and explore if the proteins can be a useful basis for discriminating these diseases.
*Methods:* In total, 124 Polish adults, including mild/moderate (M/MTB) or advanced (ATB) TB patients, and CAP patients, were enrolled in the study. The concentrations of IL-18, IL-18BP, IFN-γ, IL-37, and IP-10 in sera and *M.tb*-stimulated cultures were measured by ELISA.
*Results:* The most specific and sensitive serum proteins discriminating TB from CAP were IP-10 and IL-18BP; however, IP-10 had the highest AUC in the ROC curve for the diagnosis. Serum IP-10 and IL-18BP levels increased significantly in M/MTB or ATB groups. The IL-18BP elevation in ATB group was accompanied by an increase in IL-18. No single protein measured in *M.tb*-stimulated cultures differed TB from CAP patients.
*Conclusions:* The combined analysis of serum IL-18BP and IP-10 might be considered as an auxiliary tool in the differentiation of TB from CAP.


## 1. Introduction

According to the estimates of the World Health Organization, tuberculosis (TB) affects about 10 million people in the world and is a cause of 2 million deaths annually (WHO, 2018). Pulmonary TB is the most common form of the disease; however, the disease can develop in other organ systems such as the central nervous system, skeleton, or gastrointestinal tract.

The immunology of TB is complex and multifaceted. The control of *M.tb* infection mainly depends on the development of Th1 cell immunity involving the participation of activated macrophages, T lymphocytes, and their cytokines that affect interactions and activity of cells engaged in an antimycobacterial immune response. One of the cytokines that have been implicated in both protective and pathological processes associated with *M.tb* infection is IL-18, which is produced by a wide range of immune cells such as monocytes, macrophages, dendritic cells, epithelial cells, keratinocytes, and synovial fibroblasts as well as T and B lymphocytes (Barksby et al., 2007; Dima et al., 2015; Novick et al., 2013). Because of its property to induce IFN-γ in T cells and natural killer cells, IL-18 is considered a member of the Th1 cytokines family. The IL-18 precursor, pro-IL-18, requires cleavage to an active molecule by the intracellular caspase-1 (Dinarello, 1998; Novick et al., 2013;

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Wawrocki and Druszczynska, 2017). After cleavage, approximately 20% of the mature cytokine is secreted from macrophages/monocytes, whereas the remaining 80% of the pro-IL-18 remains unprocessed inside the cells (Dinarello et al., 2013; Sugawara et al., 2001). IL-18 binds the ligand-receptor IL-18Rα, inducing the recruitment of IL-18Rβ subunit to form a high-affinity complex, which signals through the Toll/interleukin-1 receptor (TIR) domain (Dinarello, 1999; Fields et al., 2019; Krumm et al., 2014; Plater-Zyberk et al., 2001). This signalling domain recruits the adaptor protein MyD88 that activates an NF-κB pathway and triggers a pro-inflammatory signal. The activity of IL-18 can be suppressed by extracellular interleukin 18 binding protein (IL-18BP) that binds soluble IL-18 with a higher affinity than IL-18Rα and thus prevents IL-18 binding to IL-18 receptor (Nakanishi et al., 2001; Yasuda et al., 2019). IL-37is another endogenous factor that suppresses the action of IL-18. IL-37 has high homology with IL-18 and can bind to IL-18Rα, which then forms a complex with IL-18BP, thereby reducing the activity of IL-18 (Dinarello et al., 2016; Nold et al., 2010).

IL-18 is a potent immunoregulatory cytokine with various immunological properties. It regulates the mechanisms of both innate and adaptive immunity and plays a key role in host defense (Banda et al., 2003; Biet et al., 2002; Kohno et al., 1997; Matsui et al., 1997; Okamura et al., 1995; Wawrocki et al., 2016). The primary role of IL-18 is the induction of IFN-γ production through its synergistic action either with IL-12 or IL-15, which increases the surface expression of IL-18Rβ, a signal-transducing component of the IL-18R complex (Wawrocki and Druszczynska, 2017). Additionally, IL-18 promotes proliferation and activation of NK cells, polarizes T lymphocytes towards the Th1 phenotype, enhances $CD8^+$ T cell cytolytic activity and induces the synthesis of nitric oxide (NO) as well as many cytokines and chemokines (TNF-α, GM-CSF, IL-4, IL-5, IL-9, IL-13, IL-17) (Dima et al., 2015; Dinarello et al., 2013; Gardella et al., 1999; Gutzmer et al., 2003). Although IL-18 functions as an important activator of the protective immune response against intracellular pathogens, it is also implicated in pathological processes leading to tissue damage (Wawrocki and Druszczynska, 2017). Multiple studies have shown that overproduction of IL-18 is involved in the pathogenesis of pulmonary inflammation and lung injury in mice as well as idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD) and granulomatous lung diseases in humans (Dima et al., 2015; Kitasato et al., 2004; Okamoto et al., 2002; Pechkovsky et al., 2006). The IL-18-driven inflammation has been shown to be characterized by emphysema, airway fibrosis, mucus metaplasia, and vascular remodeling of the lungs (Abdel Fattah et al., 2015; Hoshino et al., 2007; Nakajima and Owen, 2012).

Taking into account difficulties in the differentiation of pulmonary TB from non-mycobacterial community-acquired lung infections and biological activity of IL-18, we assessed serum and specific *M.tb* antigens-stimulated whole blood culture levels of IL-18 and IL-18BP in patients with active pulmonary TB having a mild/moderate (M/MTB) or advanced (ATB) form of tuberculosis and patients suffering from acute non-mycobacterial community-acquired pneumonia (CAP). We also measured serum and *M.tb*-stimulated concentration of IL-37, IFN-γ, a key cytokine in antimycobacterial immunity, and serum levels of IP-10 (IFN-γ-inducible protein 10), a chemokine mediating leukocyte recruitment and activation. The aim of the study was to assess the usefulness of combined analysis of the proteins as an auxiliary tool in the diagnosis of TB, including the disease caused by a small number of *M.tb* (paucibacillary TB), that cannot be confirmed by mycobacterial culture. Covering TB patients with mild/moderate (M/MTB) or advanced (ATB) forms of tuberculosis can provide new information on the involvement of IL-18 in the pathology of TB.

## 2. Materials and methods

### 2.1. Participants patients

In total, 124 *M. bovis* BCG-vaccinated adults of both genders, 18–81 years of age, admitted with a clinical diagnosis of lung disease to the Regional Specialized Hospital of Tuberculosis, Lung Diseases, and Rehabilitation in Tuszyn, Poland, from March 2017 to September 2018, were recruited into the study. The baseline demographic information for all participants included in the study is shown in Table 1.

**Table 1**
Participant patients characteristics.

| | TB | | | CAP |
|---|---|---|---|---|
| | Total | Mild/Moderate (M/MTB) | Advanced (ATB) | |
| N | 89 | 50 | 39 | 35 |
| Sex M/F | 48/41 | 24/26 | 24/15 | 11/24 |
| Ethnicity | Caucasian | Caucasian | Caucasian | Caucasian |
| Age | | | | |
| median | 50 | 55 | 42 | 52 |
| range | 19–81 | 23–79 | 19–81 | 19–85 |
| years (IQR) | 33–63 | 36–66 | 30–58 | 38–68 |
| BCG vaccination | 100% | 100% | 100% | 100% |
| QFT result, N (%) | | | | |
| positive | 51 (57%) | 26 (52%) | 25 (64%) | 0 (0%) |
| negative | 38 (43%) | 24 (48%) | 14 (36%) | 35 (100%) |
| *M.tb* culture, N (%) | | | | |
| positive | 49 (55%) | 27 (54%) | 22 (56%) | 0 (0%) |
| negative | 40 (45%) | 23 (43%) | 17 (44%) | 35 (100%) |
| WBC, Counts/$mm^3$ | 7200 | 6000 | 8500 | 7200 |
| RBC, Counts/$mm^3$ | 4,470,000 | 4,570,000 | 4,290,000 | 4,550,000 |
| HGB, g/dl | 13 | 13.2 | 12.8 | 13.5 |
| HCT, % | 39 | 41 | 39 | 41 |
| MCHC, g/dl | 32 | 32 | 32 | 32 |
| PLT, Counts/$mm^3$ | 286,500 | 216,500 | 342,000 | 272,000 |

Abbreviations: ATB – advanced tuberculosis patients; M/MTB – mild/moderate tuberculosis patients; CAP - non-mycobacterial, community-acquired pneumonia patients; QFT – QuantiFERON TB Gold test; WBC - white blood cells; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; MCHC - mean corpuscular hemoglobin concentration; PLT – platelets.

The final diagnosis of lung disease was established eight weeks after the admission of the patients to the hospital. All of the patients were diagnosed with extensive clinical evaluation, including clinical manifestations, TB contact history, sputum smear of acid-fast bacilli (AFB), a culture of *M.tb*, and chest radiography. This allowed classification of the patients into the following two groups: group 1, consisting of 89 patients (48 men, 41 women) diagnosed with pulmonary TB confirmed by *M.tb* sputum culture, typical clinical symptoms, characteristic features on radiographs and proper responses to anti-TB treatment (TB patients), and group 2, consisting of 35 patients (11 men, 24 women) suffering from non-mycobacterial, community-acquired pneumonia (CAP) with triple-negative *M.tb* sputum culture and no previous TB history, treated and cured with wide-range antibiotics, i.e., amoxicillin/clavulanic acid, clarithromycin, clindamycin, ceftriaxone, ciprofloxacin, doxycycline (CAP patients). Based on the extent of the lesions in the lung tissue, the TB patients were classified as having: mild/moderate TB (lesions within the unilateral lung field) (M/MTB) or advanced TB (lesions beyond the unilateral lung field) (ATB). None of the individuals had evidence of HIV infection or being treated with steroids or other immunosuppressive or anti-tubercular drugs at the time of blood sampling. The study was approved by the Ethics Committee of the University in Lodz, Poland (ethical approval number 17/KBBN-UŁ/II/2016; date 2016/11/10). Informed consent to use blood for research purposes was signed by all participants.

### 2.2. Blood samples

Blood specimens (5 ml), taken from all the patients shortly after the admission to the hospital prior to the start of the treatment, were used to prepare serum and perform a whole-blood interferon-gamma assay (QuantiFERON-TB® Gold Plus (QFT), Qiagen, Germany). The QFT assay was conducted according to the manufacturer's instructions, as described in detail previously. The results were analyzed by the QuantiFERON-TB Gold Plus Analysis Software and evaluated according to the manufacturer's criteria. The result was considered positive if the difference between the IFN-γ level in plasma incubated with TB antigen and Nil control was both ≥0.35 IU/ml and ≥25% of Nil control value.

### 2.3. IL-18, IL-18BP, IL-37, IFN-γ, IP-10 assays

The concentrations of IL-18, IL-18BP, and IL-37 in sera and in the QFT culture supernatants were determined using commercial specific enzyme-linked immunosorbent assays (Human Total IL-18 DuoSet (R&D, Minneapolis, USA), Human IL-18BPa DuoSet (R&D) and Human IL-37/IL-1F7 Duoset (R&D), Human IFN-γDuoset (R&D) and Human IP-10 Duoset ELISA (R&D)), and processed according to the manufacturer's specifications. After measuring the concentrations of both IL-18 and IL-18BP in each sample, the law of mass action was used to calculate the level of free IL-18. Single IL-18BP molecule binds a single molecule of IL-18, that interaction has a dissociation constant (Kd) of 0.4 nM. Therefore, the level of free IL-18 was calculated from the equation $x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$ where *x* is [IL-18free], b is = [IL-18BP] – [IL-18] + Kd, and c is -Kd × [IL-18] (Migliorini et al., 2010). The absorbance was measured using the Victor 2 Multi-Label Counter Microplate Reader (Wallac Oy, Turku, Finland).

### 2.4. Statistical analysis

Non-parametric tests were used to compare protein levels between the diagnostic groups: Mann Whitney *U* test for two-group comparisons and Kruskal-Wallis tests for multiple groups. Categorical variables were compared using two-tailed chi-square tests. A p-value < 0.05 was considered statistically significant. Receiver operating characteristics (ROC) curve analysis was used to determine the analytic sensitivity and specificity of each studied protein. Optimal sensitivity and specificity were estimated using the Youden's index. The likelihood ratios were used for assessing the value of performing a diagnostic test. Statistical analyses were done using Statistica 12 PL (Statsoft, Poland), MedCalc (NY, USA) and GraphPad Prism 8 (GraphPad Software, USA) software.

## 3. Results

### 3.1. Patient characteristics

The characteristics of the study participants are summarized in Table 1. The group comprised 89 patients diagnosed with active pulmonary tuberculosis (TB) and 35 patients suffering from non-mycobacterial community-acquired pneumonia (CAP). There were no differences between the studied groups regarding the age or BCG vaccination rates. The proportion of men in the TB group was significantly higher than in the CAP group ($p < 0.05$). Based on the type and extent of pulmonary lesions, TB patients were categorized as having mild/moderate TB (M/MTB; n = 50) or advanced TB (ATB; n = 39). M/MTB patients had a single lobe involved or presented unilateral involvement of two or more lobes with possible cavities reaching a diameter no greater than 4 cm, whereas ATB cases were characterized by bilateral lung changes with massive affectation and multiple cavities. Forty-nine (55%) of TB patients had a positive sputum culture for *M.tb*. The frequency of culture-confirmed TB was similar among M/MTB (54%) and ATB (56%) group. Forty (45%) of *M.tb* culture-negative TB patients were diagnosed on the basis of clinical manifestations, typical features on chest radiographs, and proper response to anti-tuberculous treatment. A positive QFT result was observed in 52% and 64% of M/MTB and ATB patients, respectively, and none of the CAP individuals. Four out of 50 (8%) M/MTB patients and 5 out of 39 (13%) ATB patients had a history of healed pulmonary TB. The percentage of underlying diseases did not differ significantly between the studied TB groups. Four out of 50 (8%) M/MTB patients and 2 out of 39 (5%) ATB patients suffered from diabetes. Similar percentages of M/MTB and ATB patients suffered from cardiovascular diseases (18% vs. 15%) and neurological diseases (4% vs. 2.5%). Chronic renal failure was diagnosed in 5 (10%) and 1 (2.5%) M/MTB and ATB patients, respectively. One person from the CAP group had sarcoidosis. There were no differences in the median values of WBC, RBC, PLT counts, and other hematological parameters between M/MTB, ATB, and CAP groups (Table 1).

### 3.2. Analysis of serum IL-18, IL-18BP, IL-37, IFN-γ, and IP-10 levels

We compared the median concentrations of IL-18 (total and free), IL-18BP, IL-37, IFN- γ, and IP-10 in the sera from the TB (M/MTB and ATB at the same time), and the CAP patients using Kruskal-Wallis tests. The levels of studied proteins measured in the sera are shown in Fig. 1a–e. The concentration of total IL-18 in the sera from the ATB patients (Me 895.63, IQR (416.28, 1146.51) pg/ml) was significantly higher ($p = 0.01$) than that found in the CAP individuals (Me 530.58, IQR (238.27, 792.12), however, similar levels of total IL-18 were observed in the CAP and M/MTB groups (Me 553.85, IQR (377.58, 830.58) pg/ml) (Fig. 1a). The serum IL-18BP concentration was comparably increased in both TB groups: ATB – Me 44.66, IQR (31.69, 70.00) ng/ml and M/MTB - Me 44.66, IQR (32.45, 66.92) ng/ml, as compared to the level of IL-18BP observed among the CAP patients (Me 35.36, IQR (23.41, 46.26) ng/ml) ($p \leq 0.01$) (Fig. 1b). There were no differences in

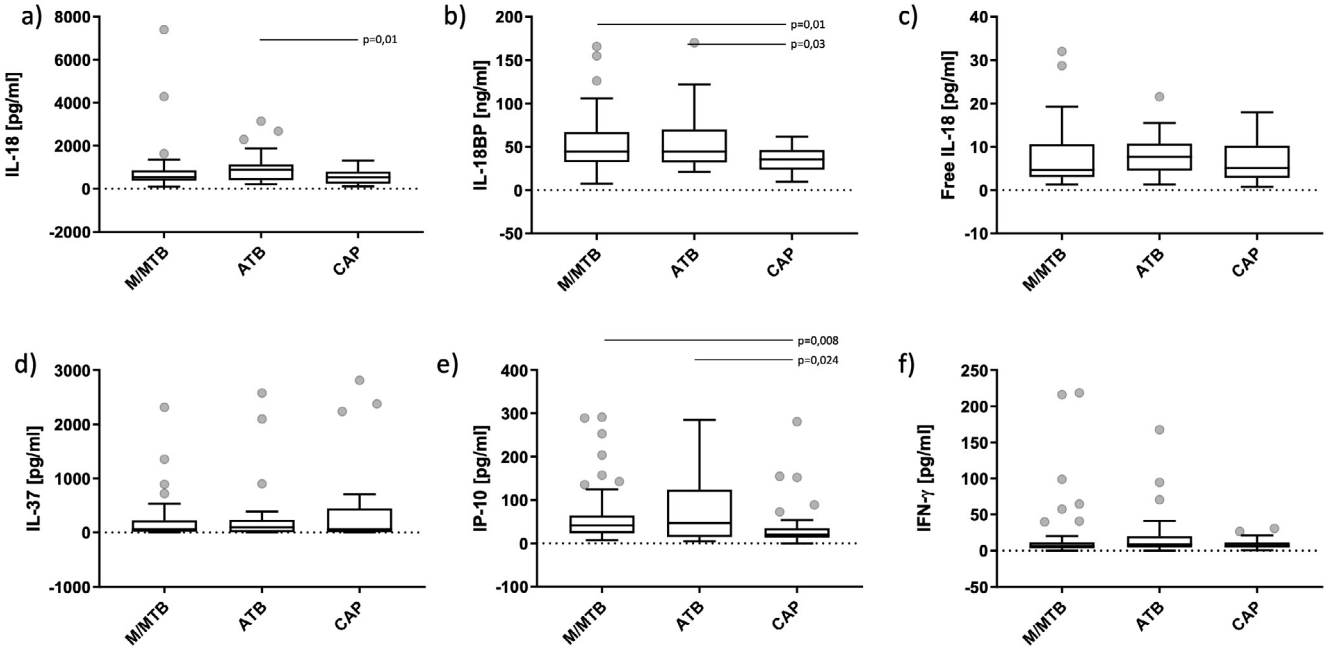


**Fig. 1.** Serum levels of total and free IL-18, IL-18BP, IL-37, IFN-γ and IP-10 in the groups of the study. Boxplots with median (horizontal line within the box), interquartile range (box limits), and extremes (whiskers) of serum levels of total IL-18 (a), IL-18BP (b), free IL-18 (c), IL-37 (d), IFN-γ (e) and IP-10 (f) in the groups of patients with mild/moderate tuberculosis (M/MTB), advanced tuberculosis (ATB) and community-acquired pneumonia (CAP).

**Table 2**
Predictive values (AUC) of individual protein levels measured in serum.

| Protein | ROC analysis | | | ROC analysis | | | ROC analysis | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total TB vs CAP | | | M/MTB vs CAP | | | ATB vs CAP | | |
| | AUC | 95% CI | p-value | AUC | 95% CI | p-value | AUC | 95% CI | p-value |
| total IL-18 | 0.635 | 0.544–0.720 | 0.017 | 0.592 | 0.480–0.697 | 0.160 | 0.685 | 0.567–0.787 | 0.002 |
| free IL-18 | 0.521 | 0.429–0.611 | 0.727 | 0.517 | 0.406–0.626 | 0.796 | 0.579 | 0.459–0.692 | 0.250 |
| IL-18BP | 0.678 | 0.588–0.759 | 0.0005 | 0.679 | 0.568–0.776 | 0.001 | 0.685 | 0.567–0.787 | 0.002 |
| IL-37 | 0.557 | 0.403–0.669 | 0.560 | 0.528 | 0.416–0.637 | 0.675 | 0.535 | 0.416–0.651 | 0.609 |
| IFN-γ | 0.533 | 0.441–0.623 | 0.546 | 0.509 | 0.399–0.620 | 0.881 | 0.597 | 0.478–0.709 | 0.141 |
| IP-10 | 0.686 | 0.596–0.766 | 0.0005 | 0.713 | 0.605–0.806 | 0.0003 | 0.659 | 0.541–0.765 | 0.013 |

the serum concentrations of free IL-18 between the M/MTB (Me 4.68, IQR (3.01, 10.29) pg/ml) and the ATB (Me 7.63, IQR (4.54, 10.78) as well as among the CAP individuals (Me 5.16, IQR (2.86, 10.29) pg/ml). (Fig. 1c). Similarly, the serum levels of IL-37 among the M/MTB, ATB, and CAP groups were comparable, measuring 66.24, IQR (15.42, 223.00) pg/ml, 102.62, IQR (6.83, 236.57) pg/ml and 63.4, IQR (6.95, 444.63) pg/ml, respectively (Fig. 1d). As shown in Fig. 1e, the serum level of IP-10 was significantly higher in both TB groups (M/MTB – Me 41.53, IQR (23.10, 62.80) pg/ml; ATB - Me 47.37, IQR (14.78, 124.28) pg/ml) than in the group of CAP patients (Me 19.60, IQR (13.51, 35.22) pg/ml. The concentration of IFN- γ observed in the sera from the M/MTB and CAP patients, was similar, measuring 6.07, IQR (3.31, 11.49) pg/ml and 6.38, IQR (4.28, 11.03) pg/ml, respectively, with a slightly elevated level of this cytokine in sera ATB patients (Me 8.59, IQR (4.53, 19.91) pg/ml.

The analysis of ROC was performed to detect the proteins with the expression levels most discriminative of the M/MTB, ATB, and CAP groups. Among the studied serum proteins, IP-10 showed the highest area under the ROC curve (AUC), namely 0.686 (95% CI 0.596–0.766, p = 0.0005) (Table 2, Fig. 2e). The AUCs of the other two proteins – IL-18BP and total IL-18 were also significantly higher than a random assignment, 0.678 (95% CI 0.588–0.759, p = 0.0005), and 0.635 (95% CI 0.544–0.720, p = 0.017), respectively. The best AUC values in M/MTB vs CAP differentiation were observed for IP-10 (AUC = 0.713, 95% CI 0.605–0.806, p = 0.0003) and IL-18BP (AUC = 0.679, 95% CI 0.568–0.776, p = 0.0019), whereas in ATB vs CAP discrimination the best AUC values in the case of IL-18BP and total IL-18 (AUC = 0.685, 95% CI 5.567–0.787, p = 0.0026) (Table 2, Fig. 2).

The most sensitive and specific serum proteins that discriminated total TB from CAP were IP-10 and IL-18BP (Table 3). The sensitivity and specificity of the ELISA assay corresponding to the cut-off of 39.11 pg IP-10/ml were 56.18% and 82.86%, respectively, whereas the cut-off of 41.40 ng IP-18BP /ml corresponded to the sensitivity of 57.30% and specificity of 71.43%. IL-18BP and IP-10 also had the highest sensitivity and specificity in differentiating the M/MTB from CAP and ATB from CAP (Table 3). Serum IFN-γ weakly differentiated the ATB from CAP (the cut-off of 17.68 pg IFN-γ/ml corresponding to the sensitivity of 32.5% and specificity of 91.43%), but it did not discriminate the M/MTB from CAP.

At the same time, it is worth noting that the analyses of IL-18, IL-18BP, IL-37, IFN-γ and IP-10 levels in the sera from the culture-positive and culture-negative M/MTB or ATB patients showed no significant differences (Table 4).

The analysis of dependence between the levels of all proteins revealed a certain correlation, that was specific for all study groups (IL-18BP and IP-10), some associations specific for a part of them

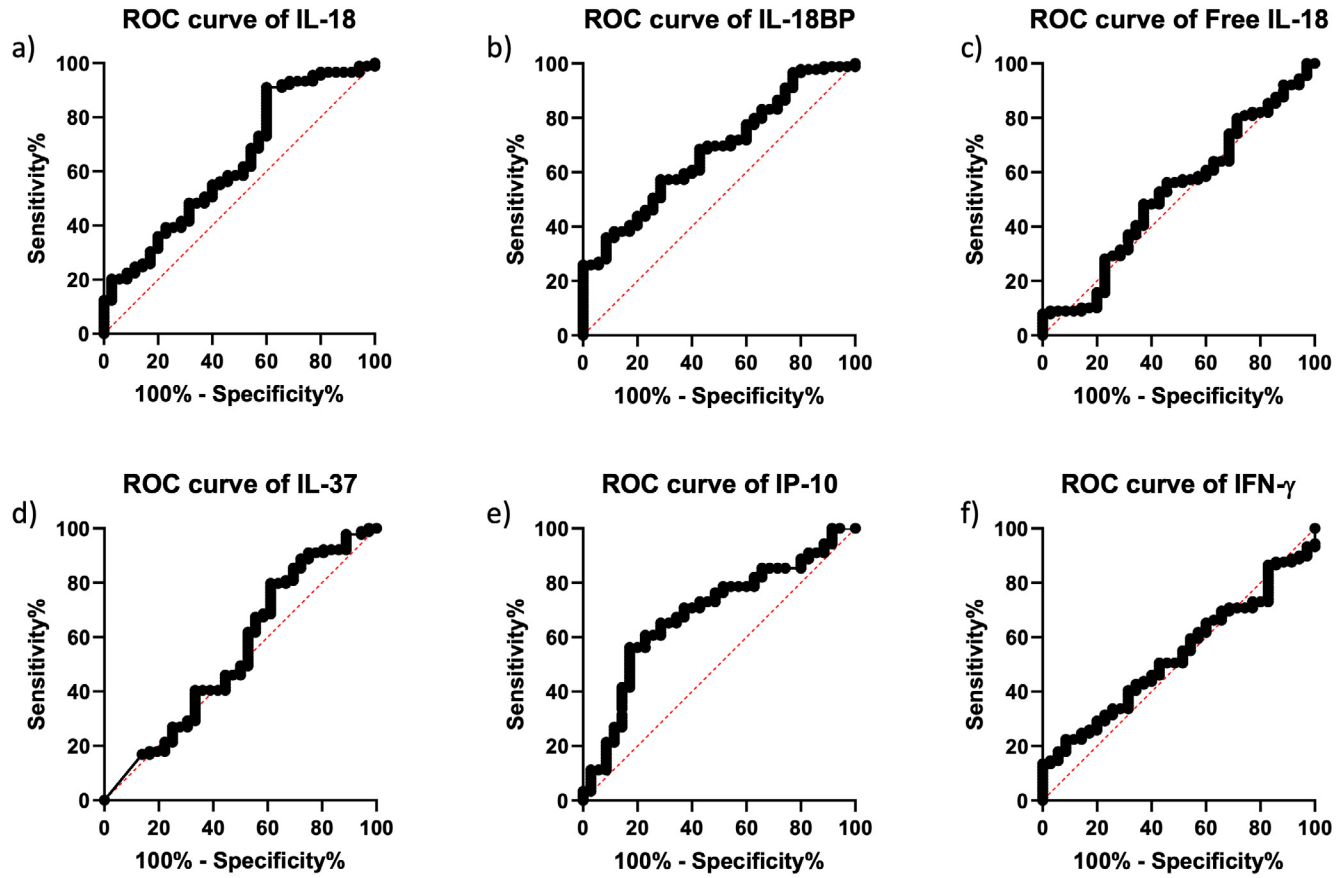


**Fig. 2.** ROC analysis of serum proteins: total IL-18 (a), IL-18BP (b), free IL-18 (c), IL-37 (d), IP-10 (e), IFN-γ (f).

**Table 3**
Sensitivity, specificity, and optimal cut-off values of individual proteins measured in serum.

| | Protein | Sensitivity (%) | Specificity (%) | Cut-off | Likelihood ratio |
|---|---|---|---|---|---|
| total TB vs CAP | total IL-18 | 91.01 | 40.00 | 281.40 pg/ml | 1.74 |
| | free IL-18 | 48.31 | 62.86 | 6.01 pg/ml | 1.06 |
| | IL-18BP | 57.30 | 71.43 | 41.40 ng/ml | 1.87 |
| | IL-37 | 79.78 | 40.00 | 259.33 pg/ml | 1.07 |
| | IFN-γ | 22.47 | 91.43 | 17.68 pg/ml | 1.30 |
| | IP-10 | 56.18 | 82.86 | 39.11 pg/ml | 1.93 |
| M/MTB vs CAP | total IL-18 | 92.00 | 40.00 | 281.40 pg/ml | 1.53 |
| | free IL-18 | 46.00 | 68.57 | 4.00 pg/ml | 1.46 |
| | IL-18BP | 42.00 | 91.43 | 53.72 ng/ml | 4.9 |
| | IL-37 | 80.00 | 37.14 | 259.33 pg/ml | 1.27 |
| | IFN-γ | 86.00 | 0.00 | 30.82 pg/ml | 0.86 |
| | IP-10 | 66.00 | 77.14 | 35.22 pg/ml | 2.89 |
| ATB vs CAP | total IL-18 | 90.00 | 40.00 | 281.40 pg/ml | 1.5 |
| | free IL-18 | 62.50 | 62.86 | 6.01 pg/ml | 1.68 |
| | IL-18BP | 62.50 | 71.43 | 41.41 ng/ml | 2.19 |
| | IL-37 | 87.50 | 28.57 | 352.64 pg/ml | 1.22 |
| | IFN-γ | 32.50 | 91.43 | 17.68 pg/ml | 3.79 |
| | IP-10 | 55.00 | 82.86 | 39.11 pg/ml | 3.21 |

(i.e., IFN-γ and IP-10 for M/MTB and ATB), and others only for one group (IL-37 and IP-10 for CAP) (Table 3). IL-18 ~ IFN-γ and IL-18BP ~ IFN-γcorrelations were specific for the ATB, but not the M/MTB group. The summary results of the correlation analysis are reported in Table 5.

### 3.3. *M.tb* antigens-stimulated IL-18, IL-18BP, and IL-37 responses

The median concentrations of IL-18, IL-18BP, and IL-37 in *M.tb* antigens-stimulated QFT supernatants are presented in Fig. 3a–e. The antigen-specific responses of the studied proteins were evaluated by subtraction of the unstimulated levels from the antigen-stimulated concentrations. There were no significant differences in the *M.tb* antigens-stimulated levels of total IL-18 among the patients with TB (M/MTB - Me 723.54, IQR (410.68, 1039.90) pg/ml, ATB - Me 806.98, IQR (452.18, 1121.67) pg/ml) and the CAP individuals (Me 688.11, IQR (332.0, 903.0) pg/ml (Fig. 3a). As shown in Fig. 3b, IL-18BP concentrations in QFT cultures from the M/MTB, ATB and CAP groups were similar, reaching 38.93, IQR (28.71, 51.28) ng/ml, 45.65, IQR (33.13, 81.79) ng/ml and

**Table 4**
Total IL-18, IL-18BP, free IL-18, IL-37, IFN-γ and IP-10 levels in the sera from the culture-positive and culture-negative M/MTB or ATB patients.

| Protein | Concentration Me (IQR) TB | | M/MTB | | ATB | |
|---|---|---|---|---|---|---|
| | Culture-positive | Culture-negative | Culture-positive | Culture-negative | Culture-positive | Culture-negative |
| total IL-18 | 719.46 (465.67–1146.10) | 490.78 (341.27–928.97) | 588.27 (444.17–963.75) | 483.72 (311.63–830.58) | 967.78 (551.64–1297.84) | 549.81 (376.73–953.65) |
| free IL-18 | 6.18 (4.23–11.26) | 4.33 (2.72–8.32) | 5.53 (3.93–12.8) | 3.61 (2.24–7.04 | 8.81 (4.66–11.18) | 6.41 (3.78–8.41) |
| IL-18BP | 44.99 (35.27–59.62) | 40.19 (30.82–77.21) | 41.72 (29.66–56.92) | 57.64 (33.22–83.17) | 46.82 (41.81–80.66) | 36.43 (26.70–50.42 |
| IL-37 | 67.32 (6.29–228.52) | 97.34 (20.43–241.17) | 67.32 (9.49–236.92) | 65.20 (15.42–223.00) | 66.23 (5.94–225.84) | 106.42 (26.97–292.27) |
| IFN-γ | 8.80 (4.46–20.69) | 5.71 (3.27–11.05) | 8.45 (3.27–16.24) | 4.70 (3.31–9.38) | 10.36 (5.46–24.69) | 5.86 (3.22–13.32) |
| IP-10 | 51.67 (26.85–80.71) | 37.14 (14.59–66.07) | 41.02 (23.10–74.00) | 42.04 (22.91–57.67) | 64.32 (31.41–130.46) | 16.92 (11.10–69.55) |

**Table 5**
Correlation and p-values between protein levels measured in serum.

| Proteins | TB | | M/MTB | | ATB | | CAP | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| IL-18 ~ IL-18BP | 0.237 | 0.025 | 0.036 | 0.801 | 0.445 | 0.004 | 0.121 | 0.488 |
| IL-18 ~ IL-37 | −0.018 | 0.867 | 0.012 | 0.929 | −0.039 | 0.812 | 0.033 | 0.848 |
| IL-18 ~ IFN-γ | 0.360 | 0.001 | 0.206 | 0.150 | 0.462 | 0.003 | −0.147 | 0.398 |
| IL-18BP ~ IL-37 | −0.146 | 0.173 | −0.159 | 0.268 | −0.135 | 0.410 | −0.101 | 0.561 |
| IL-18BP ~ IFN-γ | 0.327 | 0.002 | 0.266 | 0.061 | 0.389 | 0.014 | −0.162 | 0.350 |
| IL-37 ~ IFN-γ | −0.079 | 0.463 | −0.173 | 0.229 | 0.185 | 0.257 | 0.199 | 0.250 |
| IL-18 ~ IP-10 | 0.222 | 0.037 | 0.166 | 0.121 | 0.271 | 0.047 | 0.010 | 0.475 |
| IL-18BP ~ IP-10 | 0.597 | <0.0001 | 0.224 | 0.050 | 0.468 | 0.001 | 0.334 | 0.024 |
| IL-37 ~ IP-10 | 0.068 | 0.526 | 0.118 | 0.203 | −0.012 | 0.470 | 0.276 | 0.050 |
| IFN-γ ~ IP-10 | 0.412 | <0.0001 | 0.235 | 0.047 | 0.537 | 0.001 | 0.199 | 0.125 |
| free IL-18 ~ IL-18BP | −0.420 | <0.0001 | −0.545 | <0.0001 | −0.274 | 0.092 | −0.332 | 0.052 |
| free IL-18 ~ IL-37 | −0.143 | 0.181 | 0.066 | 0.650 | 0.015 | 0.926 | 0.0729 | 0.677 |
| free IL-18 ~ IFN-γ | 0.093 | 0.386 | 0.045 | 0.758 | 0.119 | 0.472 | −0.277 | 0.107 |
| free IL-18 ~ IP-10 | −0.004 | 0.969 | 0.084 | 0.562 | −0.096 | 0.560 | −0.223 | 0.198 |

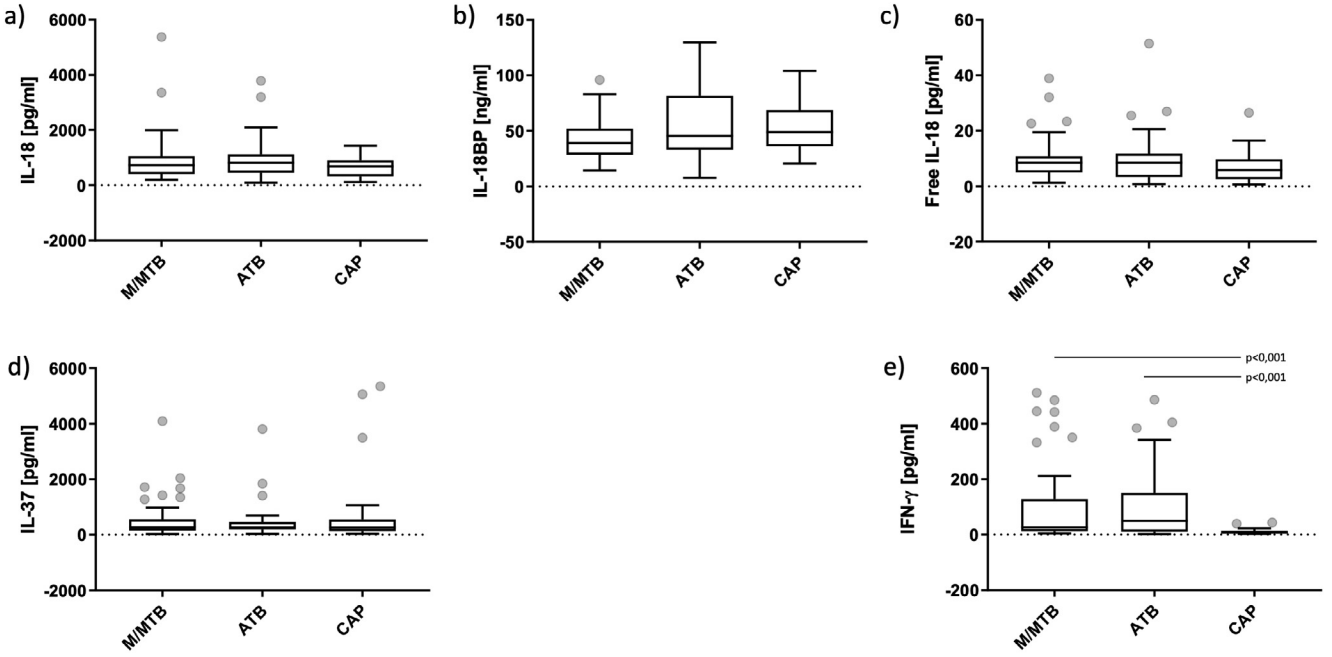


**Fig. 3.** Total IL-18, IL-18BP, free IL-18, IL-37 and IFN-γ levels in *M.tb* antigens-stimulated supernatants. Boxplots with median (horizontal line within the box), interquartile range (box limits), and extremes (whiskers) of serum levels of total IL-18 (a), IL-18BP (b), free IL-18 (c), IL-37 (d),and IFN-γ (e) in the groups of patients with mild/moderate tuberculosis (M/MTB), advanced tuberculosis (ATB) and community-acquired pneumonia (CAP).

48.68, IQR (36.05, 68.72) ng/ml, respectively. There were no significant differences in the levels of free IL-18 in *M.tb* antigens-stimulated QFT whole blood cultures among the studied groups (M/MTB - Me 8.43, IQR (4.96, 10.74) pg/ml, ATB – Me 8.46, IQR (3.28, 11.74) pg/ml, CAP – 5.80, IQR (2.50, 9.76) pg/ml) (Fig. 3c). The concentrations of IL-37 observed in QFT cultures from the M/MTB and ATB groups were comparable, measuring 274.87, IQR (156.63, 548.12) pg/ml, and 260.27, IQR (195.99, 465.44) pg/ml, respectively (Fig. 3d). The level of the cytokine in both TB groups was similar to that observed in the CAP patients (Me 257.36, IQR (128.50, 556.03) pg/ml).

The ROC analyses showed that among proteins measured in QFT supernatants, free IL-18 had the best diagnostic value in the differentiation between total TB and CAP with an AUC of 0.614 (95% CI 0.523–0.700, p = 0.045) (Table 6, Fig. 4c). IL-18BP had the highest AUC value (AUC = 0.645, 95% CI 0.533–0.745, p = 0.019) in the M/MTB vs CAP comparison, whereas free IL-18 (AUC = 0.602, 95% CI 0.483–0.714, p = 0.12) in the ATB vs CAP comparison (Table 6, Fig. 4).

IL-18BP measured in *M.tb* antigens-stimulated QFT supernatants was the most sensitive and specific cytokine to discriminate the total TB group from the CAP group (Table 7). The sensitivity and specificity of the ELISA assay corresponding to the cut-off of 45.65 ng IL-18BP/ml were 58.43% and 62.86%, respectively. Similarly, IL-18BP was characterized by the highest sensitivity and specificity in the differentiation between the M/MTB and CAP groups, whereas IL-37 was the most sensitive and specific marker in the ATB vs. CAP differentiation (Table 7).

The analyses of IL-18, IL-18BP, IL-37, IFN-γ and IP-10 levels in *M.tb* antigens-stimulated QFT supernatants from culture-positive and culture-negative M/MTB or ATB patients showed no significant differences (Table 8).

The correlation analysis between the levels of all cytokines measured in QFT supernatants showed that free IL-18 ~ IL-18BP correlation was specific for all study groups, whereas the correlation between IL-18 ~ IL-18BP was specific only for the group of M/MTB patients. The summary results of the correlation analysis are reported in Table 9.

**Table 6**
Predictive values (AUC) of individual protein levels measured in QFT supernatants.

| Protein | ROC analysis | | | ROC analysis | | | ROC analysis | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total TB vs CAP | | | M/MTB vs CAP | | | ATB vs CAP | | |
| | AUC | 95% CI | p-value | AUC | 95% CI | p-value | AUC | 95% CI | p-value |
| total IL-18 | 0.576 | 0.484–0.664 | 0.182 | 0.555 | 0.443–0.663 | 0.393 | 0.594 | 0.475–0.706 | 0.156 |
| free IL-18 | 0.614 | 0.523–0.700 | 0.045 | 0.632 | 0.520–0.734 | 0.036 | 0.602 | 0.483–0.714 | 0.120 |
| IL-18BP | 0.596 | 0.504–0.683 | 0.092 | 0.645 | 0.533–0.745 | 0.019 | 0.519 | 0.401–0.636 | 0.776 |
| IL-37 | 0.524 | 0.432–0.614 | 0.707 | 0.525 | 0.414–0.635 | 0.703 | 0.526 | 0.407–0.642 | 0.714 |

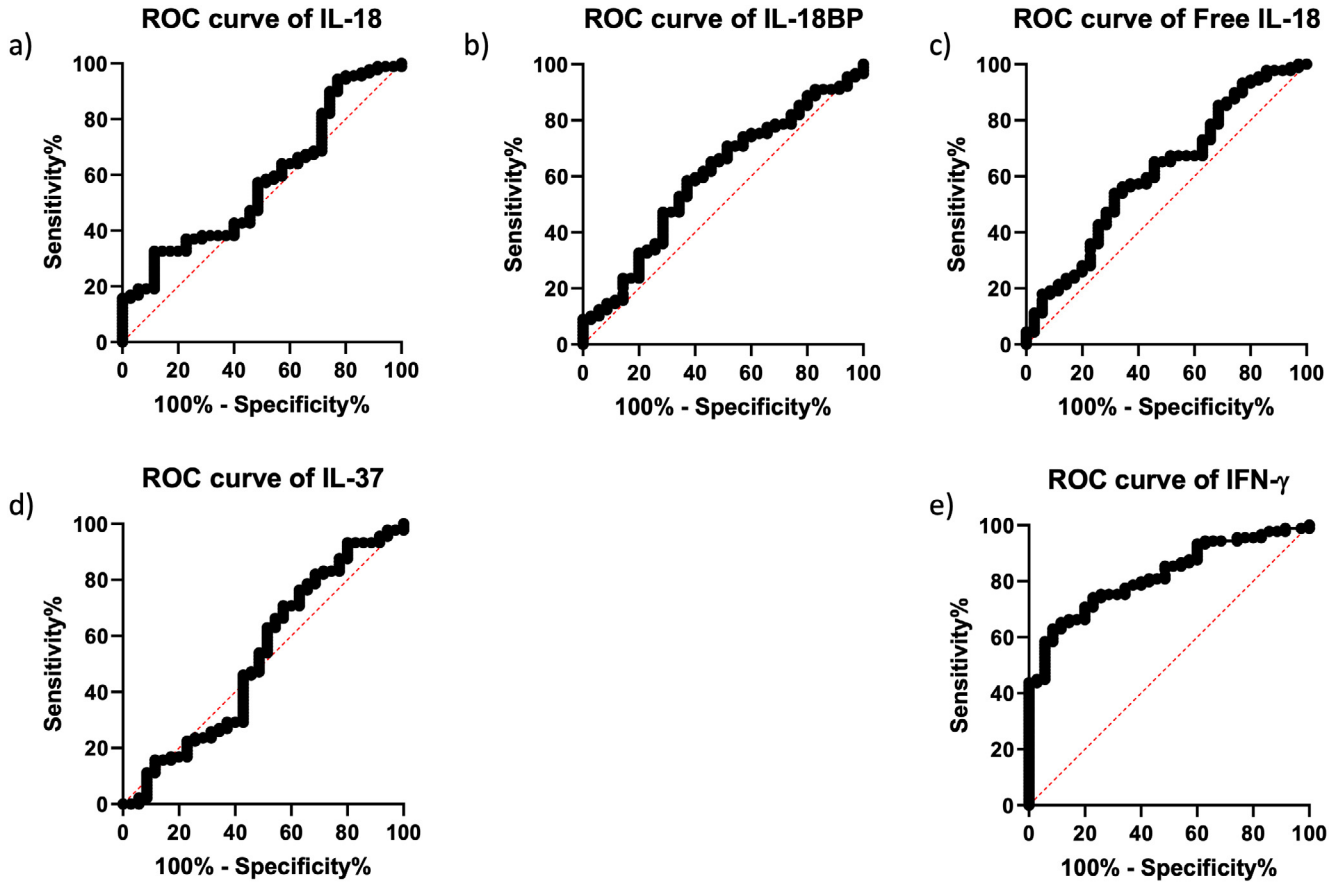


**Fig. 4.** ROC analysis of M.*tb* antigens-stimulated supernatants proteins: total IL-18 (a), IL-18BP (b), free IL-18 (c), IL-37 (d), and IFN-γ (e).

**Table 7**
Sensitivity, specificity, and optimal cut-off values of individual proteins measured in *M.tb* antigens-stimulated QFT supernatants.

| | Protein | Sensitivity (%) | Specificity (%) | Cut-off | Likelihood ratio |
|---|---|---|---|---|---|
| Total TB vs CAP | total IL-18 | 32.58 | 88.57 | 994.8 pg/ml | 1.42 |
| | free IL-18 | 53.93 | 68.57 | 8.12 pg/ml | 1.50 |
| | IL-18BP | 58.43 | 62.86 | 45.65 ng/ml | 1.35 |
| | IL-37 | 70.79 | 42.86 | 193.28 pg/ml | 0.98 |
| M/MTB vs CAP | total IL-18 | 94.00 | 22.86 | 293.77 pg/ml | 1.22 |
| | free IL-18 | 72.00 | 54.29 | 5.91 pg/ml | 1.57 |
| | IL-18BP | 64.00 | 62.86 | 44.02 ng/ml | 1.72 |
| | IL-37 | 30.00 | 57.14 | 417.59 pg/ml | 0.70 |
| ATB vs CAP | total IL-18 | 37.50 | 88.57 | 994.8 pg/ml | 3.28 |
| | free IL-18 | 47.50 | 74.29 | >9.14 pg/ml | 1.85 |
| | IL-18BP | 77.50 | 42.86 | >193.2 ng/ml | 1.36 |
| | IL-37 | 70.00 | 94.29 | >23.23 pg/ml | 12.25 |

**Table 8**
Total IL-18, IL-18BP, free IL-18, IL-37 levels in QFT supernatants from culture-positive and culture-negative M/MTB or ATB patients.

| Protein | Concentration Me (IQR) TB | | M/MTB | | ATB | |
|---|---|---|---|---|---|---|
| | Culture-positive | Culture-negative | Culture-positive | Culture-negative | Culture-positive | Culture-negative |
| total IL-18 | 773.24 (449.24–1076.76) | 749.81 (421.14–1080.21) | 744.29 (467.68–1039.90) | 626.18 (372.86–1166.25) | 876.88 (440.42–1146.72) | 765.47 (535.28–1038.75) |
| free IL-18 | 9.00 (6.16–11.85) | 6.05 (3.33–10.05) | 8.48 (6.91–10.74) | 6.69 (4.10–11.78) | 9.74 (3.70–15.22) | 5.64 (3.15–10.05) |
| IL-18BP | 39.76 (27.55–53.02) | 43.69 (33.88–64.46) | 37.08 (27.55–53.67) | 40.77 (33.71–53.67) | 42.50 (28.07–58.25) | 49.26 (41.55–81.94) |
| IL-37 | 271.50 (162.67–421.87) | 274.87 (178.02–614.73) | 271.50 (133.84–413.92) | 276.50 (181.50–778.70) | 265.93 (204.69–465.44) | 256.08 (135.87–443.72) |

**Table 9**
Correlation and p-values between the protein levels measured in QFT supernatants.

| Proteins | TB | | M/MTB | | ATB | | CAP | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| IL-18 ~ IL-18BP | 0.209 | 0.051 | 0.285 | 0.044 | 0.056 | 0.732 | 0.061 | 0.725 |
| IL-18 ~ IL-37 | −0.123 | 0.250 | −0.117 | 0.418 | −0.117 | 0.478 | 0.055 | 0.751 |
| IL-18BP ~ IL-37 | −0.071 | 0.506 | −0.100 | 0.487 | −0.029 | 0.587 | −0.241 | 0.163 |
| Free IL-18 ~ IL-18BP | −0.499 | <0.0001 | −0.358 | 0.011 | −0.623 | <0.0001 | −0.525 | 0.001 |
| Free IL-18 ~ IL-37 | −0.047 | 0.660 | 0.005 | 0.972 | −0.100 | 0.545 | −0.016 | 0.929 |

### *3.4. Cytokine ratios measured in serum or M.tb antigens-stimulated QFT supernatants*

The discriminative power of the ratios in serum or *M.tb* antigens-stimulated cultures between any two of the studied proteins (total IL-18, free IL-18, IL-18BP, IL-37, and IP-10) was analyzed in the M/MTB, ATB, and CAP groups. The highest discriminative power (of all ratios for proteins measured in serum): (1) between M /MTB and CAP was achieved by free IL-18/IP-10 ratio (AUC = 0.643) as well as IL-18BP/free IL-18 ratio (AUC = 0.615); (2) between ATB and CAP was achieved by IL-18/IL-37 ratio (AUC = 0.603) as well as free IL-18/IP-10 ratio (AUC = 0.568), (3) between M/MTB and ATB was achieved by total IL-18/IL-37 ratio (AUC = 0.606) as well as IL-18BP/IL-37 ratio (AUC = 0.602). On the other hand, the highest discriminative power (of all ratios for proteins measured in the culture supernatants): (1) between M/MTB and CAP and between ATB and CAP was achieved by IL-18BP/free IL-18 ratio (AUC = 0.669 and AUC = 0.584, respectively) as well as free IL-18/IL-37 ratio (AUC = 0.583 and AUC = 0.576, respectively); (2) between M/MTB and ATB was achieved by IL-18BP/free IL-18 ratio (AUC = 0.574) as well as IL-18BP/total IL-18 ratio (AUC = 0.562).

### *3.5. Penalized multiple logistic regression for comparison between the study groups.*

Measuring multiple protein levels in serum and cultured supernatants, we aimed to develop a linear model with the highest discriminative power between the study groups. Due to the small sample size, it was first aimed to increase discriminative power by regressing out potential confounders. Therefore, we first asked the question of whether the abundance of certain circulating cells could affect the concentration of proteins. We performed a bootstrapped (300 replicates) 5-fold cross-validation feature selection analysis based on penalized multivariate linear regression (elastic-net) to find that for the protein levels measured in serum the significant predictors were RBC, HGB, and HCT, whereas for the protein levels measured in culture supernatants the informative features were HGB and HCT. Subsequently, for each protein separately, we regressed out the effect of the selected features with

the use of standard linear models. In what follows, we trained four logistic elastic-net models (one for each group-wise comparison in one of the two compartments separately) using the above residuals as predictors. We found IL-18 and IL-18BP to be weak predictors in serum and no predictors in culture supernatants to be informative of the M/MTB versus CAP status. As far as the CAP versus ATB comparison is concerned, we found IL-18BP, free IL-18, and IL-37 in serum and IL-18 as well as IL-18BP to be associated with odds ratio in the culture supernatants. The results are summarized in Supplementary Table S1.

## 4. Discussion

In addressing the need for rapid and accurate diagnostic tests allowing the differentiation of active pulmonary TB from non-mycobacterial community-acquired pneumonia, we estimated if individual protein levels (IL-18, IL-18BP, IL-37, IFN-γ, and IP-10) and/or their ratios, might be considered auxiliary immunological biomarkers.

In a recent study, performed among TB patients and healthy volunteers, we detected an imbalance between the members of the IL-18 signalling complex in active TB, which resulted in a significant increase in the serum levels of total IL-18, IL-18BP, IFN-γ and IP-10 and a simultaneous decrease in the concentration of the anti-inflammatory IL-37 (Wawrocki et al., 2019).

In the current study, we found the potential of IL-18BP and IP-10 in the differentiation of the whole group of TB and CAP patients (Table 2). The highest discriminative power was found for IP-10 and IL-18BP measured in serum. The potential of IL-18BP and IP-10 in the differentiation of pulmonary TB and non-mycobacterial lung infections, to the best of our knowledge, had not been demonstrated before. We detected AUCs at the level of 0.686 for IP-10 and 0.678 for IL-18BP for the discrimination between TB and CAP patients.

As a naturally occurring inhibitor of IL-18, IL-18BP neutralizes the circulating IL-18. The consequences of IL-18 neutralization can include down-regulating Th1 immune response, blocking the expansion of Th17 cells, and inhibiting the infiltration of T lymphocytes into the sites of inflammation (Chiossone et al., 2012; Millward et al., 2010; Plitz et al., 2003). Significant overproduction of serum IL-18BP in the group of TB patients may be interpreted as a feedback mechanism that limits excessive IL-18 activity. However, we hypothesize that the process of modifications in the IL-18 signaling system in active TB is more complex. In the group of M/MTB patients categorized as having mild/moderate TB, the upregulated level of IL-18BP was accompanied by no visible increase in the IL-18 level. At the same time, in the group of patients with advanced TB (ATB), the upregulation of IL-18BP and IL-18 was observed simultaneously (Fig. 1A and B). As a consequence, the correlation between IL and 18 and IFN-γ as well as IL18BP and IFN-γ, was specific for the ATB but not the M/MTB group. Interestingly, the association between free IL-18 and IL-18BP was noted as specific for M/MTB group. Moreover, the highest discriminative power between M/MTB and ATB was achieved by total IL-18/IL-37 ratio (AUC = 0.606) as well as IL-18BP/IL-37 ratio (AUC = 0.602), confirming previously observed imbalance between the members of the IL-18 signaling complex in active pulmonary TB, in the study performed among TB patients and healthy volunteers(Wawrocki et al., 2019). Based on our results, we hypothesize that the elevated levels of IL-18BP were sufficient to counteract the increase in the IL-18 level in the M/MTB group but not sufficient in the ATB patients with a more advanced lung injury. This is probably due to the more efficient production of IL-18 in the ATB than in the M/MTB patients. The role of IL-18 produced by alveolar epithelial cells type II in pathomechanisms of pulmonary TB has been proposed by Pechcovsky et al.. A significant increase in the serum levels of IL-18, IFN-γ and soluble Fas in complicated TB cases as compared to uncomplicated ones was considered as a marker suggesting pulmonary TB, especially in advanced cases. Fas signaling triggers apoptosis and the production and release of mature IL-18. Biologically active IL-18 is able to enhance NK and T cell cytotoxicity and induce the production of matrix metalloproteinases, a process essential for the development of inflammatory reaction and tissue damage. Thus, the overproduction of IL-18 could explain a more advanced lung injury in the ATB patients as compared to the M/MTB group (El-Masry et al., 2007; Pechkovsky et al., 2006).

To explain the simultaneous overproduction of IL-18BP and IL-18 in the ATB patients and the selective intensification of IL-18BP in the M/MTB patients, it is worth mentioning that various isoforms of IL-18BP have different affinity for IL-18. For example, IL-18BPa and IL-18BPc effectively neutralize IL-18, while IL-18BPb and IL-18BPd isoforms lack the ability to bind and neutralize IL-18 (Kim et al., 2000). It is likely that the simultaneous increase in the IL-18BP and IL-18 levels in the ATB group, at least in part, may be a result of the preferential secretion of nonfunctional isoforms of IL-18BP. This observation may point to IL-18BP as a potential target in novel therapeutic approaches. A number of studies demonstrated the use of IL-18BP in the treatment of IL-18-associated diseases characterized by an abnormal ratio of IL-18/IL-18BP in body fluids. The administration of exogenous IL-18BP is a promising therapeutic strategy in psoriasis, experimental autoimmune encephalomyelitis, contact hypersensitivity, rheumatoid arthritis, LPS-induced shock, and atherosclerosis (Banda et al., 2003; Chiossone et al., 2012; Faggioni et al., 2001; Millward et al., 2010; Plitz et al., 2003; Schif-Zuck et al., 2005; Tak et al., 2006). Experimental data indicated that the IL-18BP-driven reduction in cell activity was dose-dependent; however, treatment with low, but not with high, doses of IL-18BP was shown to be the most effective *in vivo* (Banda et al., 2003).

The results of our study indicate a potential role of IL-18 in the pathomechanisms of pulmonary TB. It is important to note that IL-18 cannot serve as an indicator for differential diagnosis between active TB and non-mycobacterial community-acquired pneumonia, as indicated by the weak discriminative power. We show that the levels of total IL-18 in the sera from the M/MTB and CAP patients were comparable, although significantly higher in ATB patients as compared to the CAP group. Similarly, the discriminative power of the ratios analyzed between any of the two of the studied proteins measured in serum differed between the TB groups. The free IL-18/IP-10 ratio and IL-18BP/free IL-18 measured in serum was the best scoring in the discrimination analysis between M/MTB and CAP groups, whereas the highest importance scores for classification between ATB and CAP were assigned to serum total IL-18/IFN-γ and IL-18BP/IFN-γ. In contrast, IL-18BP/IP10 ratio measured in serum showed the highest discriminative power for distinguishing the entire TB group from the CAP patients. Various studies showed that the cytokines ratios might act as more discriminatory and sensitive TB indicators than assessing a single protein level (Demissie et al., 2004; Goyal et al., 2016; Joshi et al., 2015; Sun et al., 2016; Suter-Riniker et al., 2011; Wassie et al., 2008). Goyal et al. showed a potential role of the IFN-γ/IL-2 ratio in the diagnosis of extrapulmonary TB, Demissie et al. reported a discriminative power of the IL-4/IL-4δ2 in the evaluation of *M.tb* reactivation risk among latently infected individuals, whereas Wassie et al. demonstrated that IFN-γ/IL-4 and IL-4δ2/IL-4 mRNA ratios could serve as valuable markers for TB susceptibility (Demissie et al., 2004; Goyal et al., 2016; Wassie et al., 2008).

The second part of our studies was to check whether the individual and pairwise related IL-18 (total and free), IL-18BP and IL-37 levels, as well as their ratios, measured in *M.tb* specific antigen-stimulated QuantiFERON culture supernatants, might be

considered auxiliary immunological biomarkers in the differentiation between patients with active TB and patients with community-acquired pneumonia. We found no significant differences in the levels of individual proteins of the IL-18 signaling complex in *M.tb* antigens-stimulated QFT cultures among the TB patients categorized as having mild/moderate TB or advanced TB and patients with CAP. In contrast, the serum IL-18BP, IP-10, and IL-18 levels were able to differentiate between TB and CAP patients. In this way, we confirmed our earlier suggestion that the unstimulated serum biomarker performance is a better approach to diagnose patients with active TB, differentiate them from CAP patients, but also from healthy individuals with a latent *M.tb* infection and uninfected (Wawrocki et al., 2019). In the correlation analysis between levels of IL-18, IL-18 BP, and IL-37 measured in QFT supernatants, we demonstrated that free IL-18 and IL-18BP correlation was specific for all study groups. On the other hand, the correlation between total IL-18 and IL-18BP was specific for the M/MTB group, but not for the ATB group. In the interpretation of our results on the discriminative power of the levels of the IL-18 signalling complex proteins in QFT whole blood cultures (for TB and CAP patients), it should be noted that the production of cytokines by peripheral blood mononuclear cells is variable and it is influenced by many heritable and non-heritable factors (Schirmer et al., 2018). Genetic variation in individual genes and pathways responsible for cytokine production capacity can be a significant part of cytokine production variability observed among individuals. Non-heritable factors, including age, body weight, and composition of the human microbiome, can also drive variation in baseline cytokine levels. Thus, cytokine variability may have important implications for clinical practice, and the caution in interpreting the results is required to distinguish the immune response to a specific infectious agent from nonspecific inter-individual variations in cytokine responses.

Given the moderate size of the study groups, we aimed to perform a more speculative analysis in which we train an elastic-net model to detect the most robust predictors of the study groups. In this analysis, we detected only a small number of informative, in essence, the IL-18 and IL-18BP were the only significant ones in discrimination of CAP and either M/MTB or ATB cases. Therefore, we strongly believe that in order to find a comprehensive panel of proteins that are both sensitive and specific in discriminating TB and CAP, further and larger studies are required.

Because of the unpredictable and nonspecific clinical manifestation of TB, the paucibacillary TB cases can be a diagnostic challenge for physicians. In 2018, only 55% of pulmonary TB cases were bacteriologically confirmed globally (WHO, 2018). Accordingly, in this study, 55% of TB cases (52% of M/MTB and 56% of ATB) were confirmed by culture. We assessed the levels of IL-18, IL-18BP, and IL-37 in these patient groups, however, we were observing no differences in the levels of the studied proteins either in serum or *M. tb*-stimulated QFT cultures from culture-positive and culture-negative M/MTB or ATB patients.

The systematic review of publications concerning immunoenzymatic assessment of selected proteins and cytokines indicates that none of the commercial tests performed by itself provide an accurate diagnosis of TB. What is more, most of the large-scale research and meta-analysis for years focused mainly on assessing IFN-γ levels in commercial IGRA assays. They show that the assessment of the IFN-γ level alone is not a sufficient indicator to confirm *M.tb* infection. A heterogeneous methodology that does not overlap between studies limits comparability and interpretation of results. A rigorous approach to enzyme immunoassays in the diagnosis of tuberculosis in children and adults is needed. Therefore, it seems extremely important to search for new tuberculous biomarkers and evaluate intricate cytokine panels to confirm the disease, while adhering to the highest research standards. Meta-analyzes also emphasize that world trends in the diagnosis of latent TB should be changed to understand this disease better (Mandalakas et al., 2011; Rogerson et al., 2013; Steingart et al., 2011; Sun et al., 2011; Zeng et al., 2017; Zhou et al., 2015).

In conclusion, our study indicates that a combined analysis of serum IL-18BP and IP-10 could offer good classification performance and might be considered as an auxiliary tool in the differentiation of TB from CAP. At the same time, our results may enrich knowledge about the role of IL-18 in the immunopathogenesis of pulmonary TB.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.09.003.

## References

Abdel Fattah, E., Bhattacharya, A., Herron, A., Safdar, Z., Eissa, N.T., 2015. Critical role for IL-18 in spontaneous lung inflammation caused by autophagy deficiency. J. Immunol. 194, 5407–5416. https://doi.org/10.4049/jimmunol.1402277.

Banda, N.K., Vondracek, A., Kraus, D., Dinarello, C.A., Kim, S.-H., Bendele, A., Senaldi, G., Arend, W.P., 2003. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. J. Immunol. 170, 2100–2105. https://doi.org/10.4049/jimmunol.170.4.2100.

Barksby, H.E., Lea, S.R., Preshaw, P.M., Taylor, J.J., 2007. The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. Clin. Exp. Immunol. 149, 217–225. https://doi.org/10.1111/j.1365-2249.2007.03441.x.

Biet, F., Locht, C., Kremer, L., 2002. Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. J. Mol. Med. https://doi.org/10.1007/s00109-001-0307-1.

Chiossone, L., Audonnet, S., Chetaille, B., Chasson, L., Farnarier, C., Berda-Haddad, Y., Jordan, S., Koszinowski, U.H., Dalod, M., Mazodier, K., Novick, D., Dinarello, C.A., Vivier, E., Kaplanski, G., 2012. Protection from inflammatory organ damage in a murine model of hemophagocytic lymphohistiocytosis using treatment with IL-18 binding protein. Front. Immunol. 3. https://doi.org/10.3389/fimmu.2012.00239.

Demissie, A., Abebe, M., Aseffa, A., Rook, G., Fletcher, H., Zumla, A., Weldingh, K., Brock, I., Andersen, P., Doherty, T.M.VACSEL Study Group, 2004. Healthy individuals that control a latent infection with Mycobacterium tuberculosis express high levels of Th1 cytokines and the IL-4 antagonist IL-4delta2. J. Immunol. 172, 6938–6943. https://doi.org/10.4049/jimmunol.172.11.6938.

Dima, E., Koltsida, O., Katsaounou, P., Vakali, S., Koutsoukou, A., Koulouris, N.G., Rovina, N., 2015. Implication of Interleukin (IL)-18 in the pathogenesis of chronic obstructive pulmonary disease (COPD). Cytokine 74, 313–317. https://doi.org/10.1016/j.cyto.2015.04.008.

Dinarello, C.A., 1999. IL-18: AtH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J. Allergy Clin. Immunol. 103, 11–24. https://doi.org/10.1016/S0091-6749(99)70518-X.

Dinarello, C.A., 1998. Interleukin-1β, interleukin-18, and the interleukin-1β converting enzyme. Ann. N. Y. Acad. Sci., 1–11 https://doi.org/10.1111/j.1749-6632.1998.tb08307.x.

Dinarello, C.A., Nold-Petry, C., Nold, M., Fujita, M., Li, S., Kim, S., Bufler, P., 2016. Suppression of innate inflammation and immunity by interleukin-37. Eur. J. Immunol. 46, 1067–1081. https://doi.org/10.1002/eji.201545828.

Dinarello, C.A., Novick, D., Kim, S., Kaplanski, G., 2013. Interleukin-18 and IL-18 binding protein. Front. Immunol. 4, 1–10. https://doi.org/10.3389/fimmu.2013.00289.

El-Masry, S., Lotfy, M., Nasif, W., El-Kady, I., Al-Badrawy, M., 2007. Elevated serum level of interleukin (IL)-18, interferon (IFN)-γ and soluble fas in patients with pulmonary complications in Tuberculosis. Acta Microbiol. Immunol. Hung. 54, 65–77. https://doi.org/10.1556/AMicr.54.2007.1.7.

Faggioni, R., Cattley, R.C., Guo, J., Flores, S., Brown, H., Qi, M., Yin, S., Hill, D., Scully, S., Chen, C., Brankow, D., Lewis, J., Baikalov, C., Yamane, H., Meng, T., Martin, F., Hu, S., Boone, T., Senaldi, G., 2001. IL-18-binding protein protects against lipopolysaccharide- induced lethality and prevents the development of Fas/Fas ligand-mediated models of liver disease in mice. J. Immunol. 167, 5913–5920. https://doi.org/10.4049/jimmunol.167.10.5913.

Fields, J.K., Günther, S., Sundberg, E.J., 2019. Structural basis of IL-1 family cytokine signaling. Front. Immunol. https://doi.org/10.3389/fimmu.2019.01412.

Gardella, S., Andrei, C., Costigliolo, S., Poggi, A., Zocchi, M.R., Rubartelli, A., 1999. Interleukin-18 synthesis and secretion by dendritic cells are modulated by interaction with antigen-specific T cells. J. Leukoc. Biol. 66, 237–241. https://doi.org/10.1002/jlb.66.2.237.

Goyal, N., Kashyap, B., Kaur, I.R., 2016. Significance of IFN-γ/IL-2 ratio as a circulating diagnostic biomarker in extrapulmonary tuberculosis. Scand. J. Immunol. 83, 338–344. https://doi.org/10.1111/sji.12424.

Gutzmer, R., Langer, K., Mommert, S., Wittmann, M., Kapp, A., Werfel, T., 2003. Human dendritic cells express the IL-18R and are chemoattracted to IL-18. J. Immunol. 171, 6363–6371. https://doi.org/10.4049/jimmunol.171.12.6363.

Hoshino, T., Kato, S., Oka, N., Imaoka, H., Kinoshita, T., Takei, S., Kitasato, Y., Kawayama, T., Imaizumi, T., Yamada, K., Young, H.A., Aizawa, H., 2007. Pulmonary inflammation and emphysema: role of the cytokines IL-18 and IL-13. Am. J. Respir. Crit. Care Med. 176, 49–62. https://doi.org/10.1164/rccm.200603-316OC.

Joshi, L., Ponnana, M., Sivangala, R., Chelluri, L.K., Nallari, P., Penmetsa, S., Valluri, V., Gaddam, S., 2015. Evaluation of TNF-α, Il-10 and Il-6 cytokine production and their correlation with genotype variants amongst tuberculosis patients and their household contacts. PLoS ONE 10,. https://doi.org/10.1371/journal.pone.0137727 e0137727.

Kim, S.H., Eisenstein, M., Reznikov, L., Fantuzzi, G., Novick, D., Rubinstein, M., Dinarello, C.A., 2000. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc. Natl. Acad. Sci. U. S. A. 97, 1190–1195. https://doi.org/10.1073/pnas.97.3.1190.

Kitasato, Y., Hoshino, T., Okamoto, M., Kato, S., Koda, Y., Nagata, N., Kinoshita, M., Koga, H., Yoon, D.-Y., Asao, H., Ohmoto, H., Koga, T., Rikimaru, T., Aizawa, H., 2004. Enhanced expression of interleukin-18 and its receptor in idiopathic pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol. 31, 619–625. https://doi.org/10.1165/rcmb.2003-0306OC.

Kohno, K., Kataoka, J., Ohtsuki, T., Suemoto, Y., Okamoto, I., Usui, M., Ikeda, M., Kurimoto, M., 1997. IFN-γ-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. J. Immunol. 158, 1541–1550.

Krumm, B., Xiang, Y., Deng, J., 2014. Structural biology of the IL-1 superfamily: key cytokines in the regulation of immune and inflammatory responses. Protein Sci. https://doi.org/10.1002/pro.2441.

Mandalakas, A.M., Detjen, A.K., Hesseling, A.C., Benedetti, A., Menzies, D., 2011. Interferon-gamma release assays and childhood tuberculosis: Systematic review and meta-analysis. Int. J. Tuberc. Lung Dis. https://doi.org/10.5588/ijtld.10.0631.

Matsui, K., Yoshimoto, T., Tsutsui, H., Hyodo, Y., Hayashi, N., Hiroishi, K., Kawada, N., Okamura, H., Nakanishi, K., Higashino, K., 1997. Propionibacterium acnes treatment diminishes CD4+NK1.1+ T Cells but induces type I T Cells in the liver by induction of IL-12 and IL-18 production from kupffer cells. J. Immunol. 159, 97–106.

Migliorini, P., Anzilotti, C., Pratesi, F., Quattroni, P., Bargagna, M., Dinarello, C.A., Boraschi, D., 2010. Serum and urinary levels of IL-18 and its inhibitor IL-18BP in systemic lupus erythematosus. Eur. Cytokine Netw. 21, 264–271. https://doi.org/10.1684/ecn.2010.0210.

Millward, J.M., Løbner, M., Wheeler, R.D., Owens, T., 2010. Inflammation in the central nervous system and Th17 responses are inhibited by IFN-gamma-induced IL-18 binding protein. J. Immunol. 185, 2458–2466. https://doi.org/10.4049/jimmunol.0902153.

Nakajima, T., Owen, C.A., 2012. Interleukin-18: The master regulator driving destructive and remodeling processes in the lungs of patients with chronic obstructive pulmonary disease?. Am. J. Respir. Crit. Care Med. https://doi.org/10.1164/rccm.201204-0590ED.

Nakanishi, K., Yoshimoto, T., Tsutsui, H., Okamura, H., 2001. Interleukin-18 regulates both Th1 and Th2 response. Annu. Rev. Immunol. 19, 423–474. https://doi.org/10.1146/annurev.immunol.19.1.423.

Nold, M.F., Nold-Petry, C.A., Zepp, J.A., Palmer, B.E., Bufler, P., Dinarello, C.A., 2010. IL-37 is a fundamental inhibitor of innate immunity. Nat. Immunol. 11, 1014–1022. https://doi.org/10.1038/ni.1944.

Novick, D., Kim, S., Kaplanski, G., Dinarello, C.A., 2013. Interleukin-18, more than a Th1 cytokine. Semin. Immunol. 25, 439–448. https://doi.org/10.1016/j.smim.2013.10.014.

Okamoto, M., Kato, S., Oizumi, K., Kinoshita, M., Inoue, Y., Hoshino, K., Akira, S., McKenzie, A.N.J., Young, H.A., Hoshino, T., 2002. Interleukin 18 (IL-18) in synergy with IL-2 induces lethal lung injury in mice: a potential role for cytokines, chemokines, and natural killer cells in the pathogenesis of interstitial pneumonia. Blood 99, 1289–1298. https://doi.org/10.1182/blood.v99.4.1289.

Okamura, H., Tsutsui, H., Komatsu, T., Yutsudo, M., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., Akita, K., Namba, M., Tanabe, F., Konishi, K., Fukuda, S., Kurimoto, M., 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature 378, 88–91. https://doi.org/10.1038/378088a0.

Pechkovsky, D.V., Goldmann, T., Vollmer, E., Müller-Quernheim, J., Zissel, G., 2006. Interleukin-18 expression by alveolar epithelial cells type II in tuberculosis and sarcoidosis. FEMS Immunol. Med. Microbiol. 46, 30–38. https://doi.org/10.1111/j.1574-695X.2005.00013.x.

Plater-Zyberk, C., Joosten, L.A.B., Helsen, M.M.A., Sattonnet-Roche, P., Siegfried, C., Alouani, S., van de Loo, F.A.J., Graber, P., Aloni, S., Cirillo, R., Lubberts, E., Dinarello, C.A., van den Berg, W.B., Chvatchko, Y., 2001. Therapeutic effect of neutralizing endogenous IL-18 activity in the collagen-induced model of arthritis. J. Clin. Invest. 108, 1825–1832. https://doi.org/10.1172/JCI12097.

Plitz, T., Saint-Mézard, P., Satho, M., Herren, S., Waltzinger, C., de Carvalho Bittencourt, M., Kosco-Vilbois, M.H., Chvatchko, Y., 2003. IL-18 binding protein protects against contact hypersensitivity. J. Immunol. 171, 1164–1171. https://doi.org/10.4049/jimmunol.171.3.1164.

Rogerson, T.E., Chen, S., Kok, J., Hayen, A., Craig, J.C., Sud, K., Kable, K., Webster, A.C., 2013. Tests for latent tuberculosis in people with ESRD: a systematic review. Am. J. Kidney Dis. 61, 33–43. https://doi.org/10.1053/j.ajkd.2012.07.019.

Schif-Zuck, S., Westermann, J., Netzer, N., Zohar, Y., Meiron, M., Wildbaum, G., Karin, N., 2005. Targeted overexpression of IL-18 binding protein at the central nervous system overrides flexibility in functional polarization of antigen-specific Th2 cells. J. Immunol. 174, 4307–4315. https://doi.org/10.4049/jimmunol.174.7.4307.

Schirmer, M., Kumar, V., Netea, M.G., Xavier, R.J., 2018. The causes and consequences of variation in human cytokine production in health. Curr. Opin. Immunol. https://doi.org/10.1016/j.coi.2018.05.012.

Steingart, K.R., Flores, L.L., Dendukuri, N., Schiller, I., Laal, S., Ramsay, A., Hopewell, P. C., Pai, M., 2011. Commercial Serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis: an updated systematic review and Meta-Analysis. PLoS Med. https://doi.org/10.1371/journal.pmed.1001062.

Sugawara, S., Uehara, A., Nochi, T., Yamaguchi, T., Ueda, H., Sugiyama, A., Hanzawa, K., Kumagai, K., Okamura, H., Takada, H., 2001. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. J. Immunol. 167, 6568–6575. https://doi.org/10.4049/jimmunol.167.11.6568.

Sun, L., Xiao, J., Miao, Q., Feng, W.X., Wu, X.R., Yin, Q.Q., Jiao, W.W., Shen, C., Liu, F., Shen, D., Shen, A.D., 2011. Interferon gamma release assay in diagnosis of pediatric tuberculosis: a meta-analysis. FEMS Immunol. Med. Microbiol. 63, 165–173. https://doi.org/10.1111/j.1574-695X.2011.00838.x.

Sun, Q., Wei, W., Sha, W., 2016. Potential role for Mycobacterium tuberculosis specific IL-2 and IFN-γ responses in discriminating between latent infection and active disease after long-term stimulation. PLoS ONE 11,. https://doi.org/10.1371/journal.pone.0166501 e0166501.

Suter-Riniker, F., Berger, A., Mayor, D., Bittel, P., Iseli, P., Bodmer, T., 2011. Clinical significance of interleukin-2/gamma interferon ratios in Mycobacterium tuberculosis-specific T-cell signatures. Clin. Vaccine Immunol. 18, 1395–1396. https://doi.org/10.1128/CVI.05013-11.

Tak, P.P., Bacchi, M., Bertolino, M., Desson, A., 2006. Pharmacokinetics of IL-18 binding protein in healthy volunteers and subjects with rheumatoid arthritis or plaque psoriasis. Eur. J. Drug Metab. Pharmacokinet. 31, 109–116. https://doi.org/10.1007/BF03191127.

Wassie, L., Demissie, A., Aseffa, A., Abebe, M., Yamuah, L., Tilahun, H., Petros, B., Rook, G., Zumla, A., Andersen, P., Doherty, T.M., Fetcher, H., Chintu, C., Mulundu, G., Mwaba, P., McAdam, K.P.W.J., Owiafe, P., Warndorff, D., Lienhardt, C., Brookes, R., Hill, P., 2008. Ex vivo cytokine mRNA levels correlate with changing clinical status of Ethiopian TB patients and their contacts over time. PLoS ONE 3. https://doi.org/10.1371/journal.pone.0001522.

Wawrocki, S., Druszczynska, M., 2017. Inflammasomes in mycobacterium tuberculosis-driven immunity. Can. J. Infect. Dis. Med. Microbiol. https://doi.org/10.1155/2017/2309478.

Wawrocki, S., Druszczynska, M., Kowalewicz-Kulbat, M., Rudnicka, W., 2016. Interleukin 18 (IL-18) as a target for immune intervention. Acta Biochim. Pol. 63.

Wawrocki, S., Seweryn, M., Kielnierowski, G., Rudnicka, W., Wlodarczyk, M., Druszczynska, M., 2019. IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB. PLoS ONE 14. https://doi.org/10.1371/journal.pone.0225556.

World Health Organisation, 2018. Global tuberculosis report 2018.

Yasuda, K., Nakanishi, K., Tsutsui, H., 2019. Interleukin-18 in health and disease. Int. J. Mol. Sci. https://doi.org/10.3390/ijms20030649.

Zeng, N., Wan, C., Qin, J., Wu, Y., Yang, T., Shen, Y., Wen, F., Chen, L., 2017. Diagnostic value of interleukins for tuberculous pleural effusion: a systematic review and meta-analysis. BMC Pulm. Med. 17, 180. https://doi.org/10.1186/s12890-017-0530-3.

Zhou, X.X., Liu, Y.L., Zhai, K., Shi, H.Z., Tong, Z.H., 2015. Body fluid interferon-γ release assay for diagnosis of extrapulmonary tuberculosis in adults: A systematic review and meta-analysis. Sci. Rep. 5. https://doi.org/10.1038/srep15284.

# Wykaz pozostałego dorobku naukowego

## Publikacje naukowe

Druszczyńska M., Kowalewicz-Kulbat M., Maszewska A., Rudnicka K., Szpakowski P., **Wawrocki S.,** Włodarczyk M., Rudnicka W. (2015). *The balance between pro- and anti-inflammatory cytokines in the immune responses to BCG and DTwP vaccines*. Acta biochimica Polonica, 62(4), 913–921.
https://doi.org/10.18388/abp.2015_1160

**IF = 1,187; MNiSW = 15**

Ograczyk E., Kowalewicz-Kulbat M., **Wawrocki S.,** Fol M. (2015). *Immunosupresja – wymagający sprzymierzeniec na trudne czasy*. Postępy higieny i medycyny doświadczalnej, 69, 1299–1312.
https://doi.org/10.5604/17322693.1184554

**IF = 0,769; MNiSW = 15**

Druszczynska M., Kowalski K., **Wawrocki S.,** Fol M. (2017). *Diversity and functionality of mycobacterial mycolic acids in relation to host-pathogen interactions.* Current medicinal chemistry, 24(38), 4267–4278.
https://doi.org/10.2174/0929867324666170823130445

**IF = 3,469; MNiSW = 35**

Druszczynska M., Włodarczyk M., Kielnierowski G., Seweryn M., **Wawrocki S.,** Rudnicka W. (2017). *CD14-159C/T polymorphism in the development of delayed skin hypersensitivity to tuberculin.* PloS one, 12(12), e0190106.
https://doi.org/10.1371/journal.pone.0190106

**IF = 2,776; MNiSW = 35**

Druszczynska M., **Wawrocki S.,** Szewczyk R., Rudnicka W. (2017). *Mycobacteria-derived biomarkers for tuberculosis diagnosis.* The Indian journal of medical research, 146(6), 700–707.
https://doi.org/10.4103/ijmr.IJMR_1441_16

**IF = 1,508; MNiSW = 20**

Żelechowska P., Brzezińska-Błaszczyk E., Wiktorska M., Różalska S., **Wawrocki S.,** Kozłowska E., Agier J. (2019). *Adipocytokines leptin and adiponectin function as mast cell activity modulators.* Immunology, 158(1), 3–18.
https://doi.org/10.1111/imm.13090

**IF = 5,016; MNiSW = 100**

Agier J., Brzezińska-Błaszczyk E., Różalska S., Wiktorska M., **Wawrocki S.,** Żelechowska P. (2020). *β-defensin strengthens antimicrobial peritoneal mast cell response.* Journal of immunology research, 2020, 5230172.
https://doi.org/10.1155/2020/5230172

**IF = 3,327; MNiSW = 100**

**Sumaryczny IF = 18,052**

**Łączna liczba punktów MNiSW = 320**

**Podsumowanie całego dorobku**

**Sumaryczny IF = 30,985**

**Łączna liczba punktów MNiSW = 690**

*Wartość IF oraz punktację MNiSW podano zgodnie listą obowiązującą w roku publikacji*

## Publikacje popularnonaukowe

Ograczyk E., **Wawrocki S.**, Gajewski A., Kawka M., Gonciarz W., Rudnicka K., Sadowska B., Chmiela M. (2017). *Antybiotykooporność drobnoustrojów w kontekście aktualnego raportu Światowej Organizacji Zdrowia (WHO).* Laboratorium. Przegląd Ogólnopolski, (7/17).

## Komunikaty zjazdowe

### Wystąpienia ustne

**S. Wawrocki**, G. Kielnierowski, M. Druszczyńska, (2019). "Expression of IL-18, IL-18BP, IL-18R and IFN-γ genes in active tuberculosis and latent infection among the Polish population", *SIMC International Medical Congress of Silesia*, Katowice, Polska

**S. Wawrocki**, G. Kielnierowski, M. Druszczyńska, (2018). "*Mycobacterium tuberculosis* antigens-stimulated production of IL-18, IL-18BP and IL-37 in patients with pulmonary tuberculosis and healthy individuals", *18th International Congress of Young Medical Scientists*, Poznań, Polska

**S. Wawrocki**, M. Druszczyńska, (2018). "Selection of most stable reference genes for gene expression studies in latent and active tuberculosis", *IV International Conference of Cell Biology*, Kraków, Polska

**S. Wawrocki**, G. Kielnierowski, M. Druszczyńska, (2018). "Serum IL-37, IL-18 and IL-18BP levels in active tuberculosis and latent *Mycobacterim tuberculosis* infection", *SIMC International Medical Congress of Silesia*, Katowice, Polska

**S. Wawrocki**, G. Kielnierowski, M. Druszczyńska, (2018). "Serum IP-10 levels in active and latent tuberculosis", *2nd Wrocław Scientific Meetings*, Wrocław, Polska

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2017). "IL-18 secretion in relation to IL-18 gene polymorphism in *Mycobacterium tuberculosis* infected and uninfected individuals", *7th international Weigl conference*, Lwów, Ukraina

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2017). "IL-18 and IFN-γ production in cultures of blood stimulated with *Mycobacterium tuberculosis* antigens", *III International Conference of Cell Biology*, Kraków, Polska

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2017). "Evaluation of IL-18 and interleukin-18 binding protein (IL-18BP) as possible biomarkers of latent *Mycobacterium tuberculosis* infection", *SIMC International Medical Congress of Silesia*, Katowice, Polska

**S. Wawrocki**, A. Lizińczyk, J. Olszewicz, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2017). "IL-18 i IL-18BP w aktywnym zakażeniu *Mycobacterium tuberculosis"*, *III Toruńskie Sympozjum Doktorantów Nauk Przyrodniczych,* Toruń, Polska

K. Krawczyk, M. Fol, M. Druszczyńska, M. Włodarczyk, W. Rudnicka, E. Ograczyk, **S. Wawrocki**, M. Kowalewicz-Kulbat (2016). "Characteristics of memory T lymphocytes isolated from immunized with *Mycobacterium tuberculosis* mice in the model of cyclophosphamide-induced immunosuppression", the *BioChemMed Session*, Gdańsk, Polska

**Plakaty**

M. Druszczynska, **S. Wawrocki**, R. Szewczyk, A. Pankowska, (2018). „Profiling the children's immune response to *Mycobacterium tuberculosis* by cytokine array", *ASM Microbe 2019*, San Francisco, USA

**S. Wawrocki**, G. Kielnierowski, M. Druszczyńska, (2018). "IL-18, IL-18BP, IL-37 and IP-10 levels in severe and non-severe cases of pulmonary tuberculosis (TB)", *5th European Congress of Immunology 2018*, Amsterdam, Holandia

M. Druszczynska, **S. Wawrocki**, M. Baranowska, A. Pankowska, M. Kowalewska-Pietrzak, (2018). „Th17 cytokine profile in active and latent childhood tuberculosis", *European Congress of Immunology 2018*, Amsterdam, Holandia

**S. Wawrocki**, G. Kielnierowski, M. Druszczyńska, (2018). "Cytokiny rodziny IL-1 w zakażeniu *Mycobacterium tuberculosis*", *II Sesja Młodych Mikrobiologów Środowiska Łódzkiego*, Łódź, Polska

**S. Wawrocki**, M. Baranowska, A. Pankowska, M. Druszczyńska, (2018). "IFN-γ inducible protein-10 (IP-10) in childhood pulmonary TB in BCG-vaccinated population", *Young Researchers in Life Sciences,* Paryż, Francja

**S. Wawrocki,** M. Druszczyńska, (2018). "Rola inflamasomów w zakażeniu *Mycobacterium tuberculosis*", *IV Toruńskie Sympozjum Doktorantów Nauk Przyrodniczych,* Toruń, Polska

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2017). "Interleukina 18 (IL-18) oraz wiążące ją białko (IL-18BP) w odpowiedzi odpornościowej na prątki *Mycobacterium tuberculosis*", *PTM wczoraj – dziś – jutro*, Kraków, Polska

E. Ograczyk, M. Włodarczyk, M. Druszczyńska, M. Kowalewicz-Kulbat**, S. Wawrocki**, M. Fol, (2017). "Optimization of mouse cyclophosphamide-induced immunosuppression model", *IV „Microbiology in Health Care and Environmental Protection" - MIKROBIOT 2017*, Łódź, Polska

**S. Wawrocki**, J. Olszewicz, A. Lizińczyk, M. Druszczyńska, (2017). "IL-18 and IL-18BP as markers of active and latent tuberculosis", *IV „Microbiology in Health Care and Environmental Protection" - MIKROBIOT 2017*, Łódź, Polska

J. Agier, **S. Wawrocki**, E. Brzezińska-Błaszczyk, (2017). "Katelicydyna LL-37 w infekcji *Mycobacterium tuberculosis*", *I Ogólnopolskie Sympozjum Nauk Przyrodniczo-Rolniczych*, Poznań, Polska

**S. Wawrocki**, J. Agier, M. Druszczyńska, (2017). "Aktywność IL-18 i IL-18BP w organizmie człowieka", *I Ogólnopolskie Sympozjum Nauk Przyrodniczo-Rolniczych*, Poznań, Polska

J. Agier, **S. Wawrocki**, E. Kozłowska, P. Żelechowska, (2017). "Peptydy przeciwdrobnoustrojowe w infekcji *Mycobacterium tuberculosis*", *III Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen 2017*, Łódź, Polska

**S. Wawrocki**, M. Druszczyńska (2017). "Optymalizacja warunków qPCR dla oceny ekspresji genu IL-18 oraz białka (IL-18BP) i receptora ją wiążącego (IL-18R)", *III Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen 2017*, Łódź, Polska

E. Ograczyk, M. Włodarczyk, **S. Wawrocki**, M. Kowalewicz-Kulbat, M. Druszczyńska, M. Fol, (2016). "The comparison of Baxter and Sigma Cyclophosphamide on selected biological parameters on mice C57BL/6", *BioChemMed Session*, Gdańsk, Polska

**S. Wawrocki**, M. Włodarczyk, G. Kilenierowski, L. Zhurawska, W. Rudnicka, M. Druszczyńska (2016). "Association of cytokine gene polymorphism with pulmonary tuberculosis in adults in Polska", *BioChemMed Session*, Gdańsk, Polska

E. Ograczyk, **S. Wawrocki**, M. Druszczyńska, M. Włodarczyk, W. Rudnicka, M. Fol, (2016). "Reakcje odpornościowe na prątki *M. bovis* BCG modyfikowane przez cyklofosfamid (CTX) u myszy C57BL/6", *XXVIII Zjazd Polskiego Towarzystwa Mikrobiologów,* Bydgoszcz, Polska

**S. Wawrocki**, E. Ograczyk, M. Druszczyńska, M. Kowalewicz-Kulbat, W. Rudnicka, M Fol, (2016). "IL-18 w odpowiedzi komórkowej na prątki *M. bovis* BCG u myszy immunokompetentnych oraz z immunosupresją wywołaną działaniem cyklofosfamidu (CTX)", *XXVIII Zjazd Polskiego Towarzystwa Mikrobiologów,* Bydgoszcz, Polska

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2016). "Polimorfizm genu IL-18 (-607C/A) w aktywnym i utajonym zakażeniu prątkami *Mycobacterium Tuberculosis*", *II Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen 2016*, Łódź, Polska

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2016). "Polymorphism of the promoter region of IL-18 encoding gene in active and latent infection with *Mycobacterium tuberculosis*", *V Wrocław Conference of Technical and Strict Technical Students*, Wrocław, Polska

**S. Wawrocki**, E. Ograczyk, L. Zhuravska, M. Druszczyńska, W. Rudnicka, (2015). "IL-17 levels in stimulated with specific *Mycobacterium tuberculosis* antigens whole blood cultures from children with active and latent TB", *VII International Conference of Biotechnology Students, XVII National Academic Seminar of Biotechnology Students*, Poznań, Polska

E. Ograczyk, **S. Wawrocki**, M. Kowalewicz-Kulbat, M. Druszczyńska, M. Fol, (2015). "The effect of the cyclophosphamide on mice C57BL/6 immunized with BCG", *VII International Conference of Biotechnology Students, XVII National Academic Seminar of Biotechnology Students*, Poznań, Polska

**S. Wawrocki**, M. Włodarczyk, W. Rudnicka, M. Druszczyńska, (2015). "Produkcja IL-17 w stymulowanych antygenami mykobakterii hodowlach krwi pacjentów z gruźlicą i zdrowych ochotników*", III Edycja Konferencji Mikrobiologia w Medycynie, Przemyśle i Ochronie Środowiska*, Łódź, Polska

E. Ograczyk, **S. Wawrocki**, M. Fol, M. Druszczyńska, (2015). "Morfologia krwi obwodowej myszy C57BL/6 poddawanych działaniu cyklofosfamidu (CTX)", *I Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen 2015*, Łódź, Polska

M. Włodarczyk, W. Rudnicka, B. Janiszewska-Drobińska, **S. Wawrocki**, D. Lenart, M. Druszczyńska, (2014). "Cytokine response to antigens of mycobacteria in cultures of whole blood", *15th Congress of the Polish Society of Experimental and Clinical Immunology*, Wrocław, Polska

## Nagrodzone wystąpienia konferencyjne

### Wystąpienia ustne

*SIMC International Medical Congress of Silesia,* Katowice, 2019

*18th International Congress of Young Medical Scientists,* Poznań, 2018

*SIMC International Medical Congress of Silesia,* Katowice, 2018

*III Toruńskie Sympozjum Doktorantów Nauk Przyrodniczych,* Toruń, 2017

*SIMC International Medical Congress of Silesia,* Katowice, 2017

### Plakaty

*XXVIII Zjazd Polskiego Towarzystwa Mikrobiologów,* Bydgoszcz, 2016

*BioChemMed Session,* Gdańsk, 2016

# Oświadczenia współautorów o udziale w publikacjach

Oświadczenie o udziale w publikacjach

**Mgr Sebastian Wawrocki**

Katedra Immunologii i Biologii Infekcyjnej,

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e-mail: sebastian.wawrocki@biol.uni.lodz.pl

1. **Wawrocki S.,** Druszczyńska M., Kowalewicz-Kulbat M., Rudnicka W. (2016). *Interleukin 18 (IL-18) as a target for immune intervention*. Acta Biochimica Polonica, 63(1), 59–63.

   *Oświadczam, że mój udział w ww. publikacji wynosi* ***30%****, który obejmował współudział w tworzeniu koncepcji pracy, przygotowaniu manuskryptu i rycin, a także edycji pracy.*

   .....
   podpis współautora

2. **Wawrocki S.,** Druszczyńska M. (2017). *Inflammasomes in Mycobacterium tuberculosis-driven immunity*. Canadian Journal of Infectious Diseases and Medical Microbiology, 2017, 2309478.

   *Oświadczam, że mój udział w ww. publikacji wynosi* ***80%****, który obejmował współudział w tworzeniu koncepcji pracy, przygotowniu manuskryptu i rycin, a także edycji pracy.*

   .....
   podpis współautora

3. **Wawrocki S.,** Kielnierowski G., Rudnicka W., Druszczyńska M. (2019a). *Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population*. Acta Biochimica Polonica, 66(3), 337–342.

   *Oświadczam, że mój udział w ww. publikacji wynosi* ***80%****, który obejmował współudział w tworzeniu koncepcji pracy, wykonaniu doświadczeń, analizie wyników i przygotowaniu manuskryptu.*

   .....
   podpis współautora

4. **Wawrocki S.,** Seweryn M., Kielnierowski G., Rudnicka W., Włodarczyk M., Druszczyńska M. (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.

*Oświadczam, że mój udział w ww. publikacji wynosi* ***60%****, który obejmował współudział w tworzeniu koncepcji pracy, wykonaniu doświadczeń, analizie wyników i przygotowaniu manuskryptu.*

Wawrocki.....
podpis współautora

5. **Wawrocki S.,** Kielnierowski G., Rudnicka W., Seweryn M., Druszczyńska M. (2020b). *Interleukin-18, functional IL-18 receptor and IL-18 binding protein expression in active and latent tuberculosis*. Pathogens, 9(6), 451.

*Oświadczam, że mój udział w ww. publikacji wynosi* ***70%****, który obejmował współudział w tworzeniu koncepcji pracy, wykonaniu doświadczeń, analizie wyników i przygotowaniu manuskryptu.*

Wawrocki.....
podpis współautora

6. **Wawrocki S.,** Seweryn M., Kielnierowski G., Rudnicka W., Druszczyńska M. (2020a). *IL-18 and related function proteins associated with tuberculosis severity and screening for active TB among patients with non-mycobacterial community-acquired pneumonia (CAP)*. Saudi Journal of Biological Sciences, 27(11), 3035–3045.

*Oświadczam, że mój udział w ww. publikacji wynosi* ***60%****, który obejmował współudział w tworzeniu koncepcji pracy, wykonaniu doświadczeń, analizie wyników i przygotowaniu manuskryptu.*

Wawrocki.....
podpis współautora

Oświadczenie o udziale w publikacjach

**Dr hab. Magdalena Druszczyńska, prof. UŁ**

Katedra Immunologii i Biologii Infekcyjnej,

Wydział Biologii i Ochrony Środowiska,

Uniwersytet Łódzki, ul. Banacha 12/16, 90-237 Łódź

e-mail: magdalena.druszczynska@biol.uni.lodz.pl

1. Wawrocki S., **Druszczyńska M.,** Kowalewicz-Kulbat M., Rudnicka W. (2016). *Interleukin 18 (IL-18) as a target for immune intervention*. Acta Biochimica Polonica, 63(1), 59–63.

   *Oświadczam, że mój udział w ww. publikacji wynosi **30%**, który obejmował współudział w tworzeniu koncepcji pracy i przygotowaniu manuskryptu.*

   .....
   podpis współautora

2. Wawrocki S., **Druszczyńska M.** (2017). *Inflammasomes in Mycobacterium tuberculosis-driven immunity.* Canadian Journal of Infectious Diseases and Medical Microbiology, 2017, 2309478.

   *Oświadczam, że mój udział w ww. publikacji wynosi **20%**, który obejmował współudział w tworzeniu koncepcji pracy i przygotowaniu manuskryptu.*

   .....
   podpis współautora

3. Wawrocki S., Kielnierowski G., Rudnicka W., **Druszczyńska M.** (2019a). *Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population*. Acta Biochimica Polonica, 66(3), 337–342.

   *Oświadczam, że mój udział w ww. publikacji wynosi **10%**, który obejmował współudział w tworzeniu koncepcji pracy, nadzór nad realizacją badań, ocenę postępów pracy, uczestnictwo w interpretacji wyników i przygotowanie manuskryptu.*

   .....
   podpis współautora

4. Wawrocki S., Seweryn M., Kielnierowski G., Rudnicka W., Włodarczyk M., **Druszczyńska M.** (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.

*Oświadczam, że mój udział w ww. publikacji wynosi **14%**, który obejmował współudział w tworzeniu koncepcji pracy, nadzór nad realizacją badań, ocenę postępów pracy, uczestnictwo w interpretacji wyników i przygotowanie manuskryptu.*

MD Murphie
.....
podpis współautora

5. Wawrocki S., Kielnierowski G., Rudnicka W., Seweryn M., **Druszczyńska M.** (2020b). *Interleukin-18, functional IL-18 receptor and IL-18 binding protein expression in active and latent tuberculosis*. Pathogens, 9(6), 451.

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*Oświadczam, że mój udział w ww. publikacji wynosi **15%**, który obejmował współudział w tworzeniu koncepcji pracy, nadzór nad realizacją badań, ocenę postępów pracy, uczestnictwo w interpretacji wyników i przygotowanie manuskryptu.*

MD Murphie
.....
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Oświadczenie o udziale w publikacjach

**Prof. dr hab. Wiesława Rudnicka**

Katedra Immunologii i Biologii Infekcyjnej,

Wydział Biologii i Ochrony Środowiska,

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**1.** Wawrocki S., Druszczyńska M., Kowalewicz-Kulbat M., **Rudnicka W.** (2016). *Interleukin 18 (IL-18) as a target for immune intervention*. Acta Biochimica Polonica, 63(1), 59–63.

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podpis współautora

**2.** Wawrocki S., Kielnierowski G., **Rudnicka W.,** Druszczyńska M. (2019a). *Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population*. Acta Biochimica Polonica, 66(3), 337–342.

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podpis współautora

**3.** Wawrocki S., Seweryn M., Kielnierowski G., **Rudnicka W.,** Włodarczyk M., Druszczyńska M. (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.

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podpis współautora

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.....
podpis współautora

5. Wawrocki S., Seweryn M., Kielnierowski G., **Rudnicka W.,** Druszczyńska M. (2020a). *IL-18 and related function proteins associated with tuberculosis severity and screening for active TB among patients with non-mycobacterial community-acquired pneumonia (CAP)*. Saudi Journal of Biological Sciences, 27(11), 3035–3045.

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.....
podpis współautora

Oświadczenie o udziale w publikacjach

**Lek. Grzegorz Kielnierowski**

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e-mail: kielnier@o2.pl

1. Wawrocki S., **Kielnierowski G.,** Rudnicka W., Druszczyńska M. (2019a). *Lack of significant effect of interleukin-18 gene variants on tuberculosis susceptibility in the Polish population*. Acta Biochimica Polonica, 66(3), 337–342.

   *Oświadczam, że mój udział w ww. publikacji wynosi **5%**, który obejmował pozyskanie materiału biologicznego i klasyfikację pacjentów.*

   G. Kielnierowski
   podpis współautora

2. Wawrocki S., Seweryn M., **Kielnierowski G.,** Rudnicka W., Włodarczyk M., Druszczyńska M. (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.

   *Oświadczam, że mój udział w ww. publikacji wynosi **4%**, który obejmował pozyskanie materiału biologicznego i klasyfikację pacjentów.*

   G. Kielnierowski
   podpis współautora

3. Wawrocki S., **Kielnierowski G.,** Rudnicka W., Seweryn M., Druszczyńska M. (2020b). *Interleukin-18, functional IL-18 receptor and IL-18 binding protein expression in active and latent tuberculosis*. Pathogens, 9(6), 451.

   *Oświadczam, że mój udział w ww. publikacji wynosi **5%**, który obejmował pozyskanie materiału biologicznego i klasyfikację pacjentów.*

   G. Kielnierowski
   podpis współautora

4. Wawrocki S., Seweryn M., **Kielnierowski G.,** Rudnicka W., Druszczyńska M. (2020a). *IL-18 and related function proteins associated with tuberculosis severity and screening for active TB among patients with non-mycobacterial community-acquired pneumonia (CAP).* Saudi Journal of Biological Sciences, 27(11), 3035–3045.

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.....

podpis współautora

Oświadczenie o udziale w publikacjach

**Dr Michał Seweryn**

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1. Wawrocki S., **Seweryn M.,** Kielnierowski G., Rudnicka W., Włodarczyk M., Druszczyńska M. (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.

   *Oświadczam, że mój udział w ww. publikacji wynosi **14%**, który obejmował analizę statystyczną i wizualizację wyników, a także udział w tworzeniu manuskryptu.*

   .....
   podpis współautora

2. Wawrocki S., Kielnierowski G., Rudnicka W., **Seweryn M.,** Druszczyńska M. (2020b). *Interleukin-18, functional IL-18 receptor and IL-18 binding protein expression in active and latent tuberculosis*. Pathogens, 9(6), 451.

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   .....
   podpis współautora

3. Wawrocki S., **Seweryn M.,** Kielnierowski G., Rudnicka W., Druszczyńska M. (2020a). *IL-18 and related function proteins associated with tuberculosis severity and screening for active TB among patients with non-mycobacterial community-acquired pneumonia (CAP)*. Saudi Journal of Biological Sciences, 27(11), 3035–3045.

   *Oświadczam, że mój udział w ww. publikacji wynosi **15%**, który obejmował analizę statystyczną i wizualizację wyników, a także udział w tworzeniu manuskryptu.*

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**1.** Wawrocki S., Druszczyńska M., **Kowalewicz-Kulbat M.,** Rudnicka W. (2016). *Interleukin 18 (IL-18) as a target for immune intervention*. Acta Biochimica Polonica, 63(1), 59–63.

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1. Wawrocki S., Seweryn M., Kielnierowski G., Rudnicka W., **Włodarczyk M.,** Druszczyńska M. (2019b). *IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB*. PLoS One, 14(12), e0225556.

*Oświadczam, że mój udział w ww. publikacji wynosi* ***3%****, który obejmował współudział w wizualizacji wyników.*

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