

The Lancet Microbe

Inflammatory profile of HIV uninfected and co-infected patients with tuberculosis: a prospective cohort study and immunological network analysis --Manuscript Draft--

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Abstract:	Background Human immunodeficiency virus type 1 (HIV-1) mediated dysregulation of the immune response to tuberculosis (TB) and its effect on the response to antitubercular treatment (ATT) is incompletely understood. Our aim was to perform an in-depth analysis of the inflammatory profile of HIV-1-uninfected and co-infected TB patients undergoing ATT, with sub-analysis of the effect of antiretroviral therapy and HIV-1 viraemia in the latter group. Methods An extensive panel of inflammatory markers were measured in plasma of a prospective patient cohort undergoing ATT at Khayelitsha Site B Clinic, South Africa between March 2013 and July 2014. Plasma samples were collected at baseline (1-5 days after commencing ATT), week 8 and week 20 of ATT. We applied network and multivariate analysis to investigate the dynamic inflammatory profile of these patients in relation to ATT and by HIV status. Findings HIV-1 status markedly influenced the inflammatory profile regardless of ATT duration. HIV-1 viral load emerged as a major factor driving differential inflammatory marker expression and impacting on correlation profiles observed in the HIV-1 co-infected group. Unexpectedly, IL-17A emerged as a key correlate of HIV-1 induced inflammation during HIV-TB co-infection. We validated these findings in a second cohort of hospitalized HIV-TB co-infected patients where the number of statistically significant correlations with IL-17A in network analysis predicted mortality.

Interpretation

Our findings demonstrate the effect of HIV-1 co-infection on the complexity of plasma inflammatory profiles in TB patients. Through network analysis we identified IL-17A as an important node in HIV-TB co-infection, thus implicating this cytokine's capacity to correlate with, and regulate, other inflammatory markers. Further mechanistic studies are thus required to identify specific IL-17A related inflammatory pathways mediating immunopathology in HIV-TB co-infection, which may illuminate targets for future host directed therapies.

Manuscript reference number: THELANCETMICROBE-D-20-00039R2

Title: Inflammatory profile of HIV uninfected and co-infected patients with tuberculosis: a prospective cohort study and immunological network analysis

Dear Robert and Bruno,

I hope you are well and that you had a nice break over the end of the year holidays.

Thank you very much for the revisions you made to your paper submitted to *The Lancet Microbe*. You have responded thoroughly to all the points, the methods are now very clear and comprehensive, and overall the paper reads very well. The only issue is that it is too long: I realise this is partly due to our requests to add information, so we have identified parts of the text that could be either moved to the appendix or summarised/made more succinct. I have highlighted these sections in the attached PDF (from p 23 of the document) and have annotated it with suggestions of shortened text. I have also been in touch with the post-acceptance editing and production team and due to space constraints we will need to move some of the figure panels to the appendix, unfortunately. I have detailed some suggestions below, but would be happy to consider alternatives you might propose.

I am sorry to have yet some more requests, but I would like to reassure you that from the point of view of scientific content, the manuscript is ready for acceptance and it is really just a matter of getting it within the word and space limits.

I look forward to receiving a shortened version.

Kind regards,

Elena

Dr. Elena Dalla Vecchia
Senior Editor, *The Lancet Microbe*
Email: e.dallavecchia@lancet.com

EDITORS' SPECIFIC POINTS:

1. Length of the manuscript: the word limit for research articles is 3500 words, and although we have a degree of flexibility on this, 6100 words is beyond what we can accept (ideally around 4000, but we could stretch this to a maximum of 4500). I realise the length increased drastically in response to my comments, and we appreciate the additional content (some of which is really essential in understanding what was done), but some parts will need to be moved to the appendix and/or summarised in the main text. I am including an annotated PDF of your manuscript with highlighted sections that can be removed or moved to the appendix and I have also made some suggestions on alternative (shorter) text that could be included. You might need to identify some other parts to cut down on the length a bit.

We thank the editor for the helpful suggestions which have been incorporated. The manuscript has been shortened accordingly, word count is now 4379.

2. Appendix: please just keep two appendix files:
 - a. Appendix 1_Final (xlsx): please ensure it is cited in the main text (ok for it to be cited in the end matter, in the Data sharing statement)

We have done so.

- b. Supplementary Material_21DEC2020: this will need to contain
 1. the supplementary methods
 2. (including what is currently in the Appendix 2 [docx] file and the additions from the main text, to reduce its length),
 3. supplementary figures and tables (as they are now is fine),
 4. strobe statement (this is not strictly necessary, it was helpful for us in checking reporting), and
 5. study protocol (if this is available online, you could alternatively provide a weblink in the text).
 6. Please also include a table of content page at the beginning and ensure that all pages are numbered. Once this document is ready, please convert it to PDF and check it carefully, as we do not edit supplementary material post-acceptance.

We have done so.

3. Figures 1-4: I have been in touch with a colleague from the post-acceptance editing and production team, and regretfully it seems as though there are still too many figures. Even though we allow up to 5 non-text items, your figures are very complex and contain a lot of panels, so as they are it would be difficult to fit them in our layout.

We have some suggestions, but I'd be happy to discuss other options with you:

- a. Figure 1: this figure essentially incorporates what could be three separate figures, according to our standard layouts (ie, panels A+B; panel C; and panels D-F), so combined with the other figures the maximum number of non-text items would be exceeded. As B provides similar (though summarised and simplified) information to panel A, panel A could maybe be moved to the appendix. We would also suggest moving panels D and F to the appendix. So essentially keeping **in figure 1 panels B, C, and E**, and just describing in the text the results of A, D, and F, and referring to the appendix.
- b. Figure 2: As for figure 1, maybe panel A could be moved to the appendix, **keeping panel B** that summarises the results of the expression data more concisely?
- c. Figure 3: Maybe panel A could be moved to the appendix. As you have exemplified in figures 1 and 2 the node diagrams, describing in the text the differences in node density could be enough. The remaining panels would then provide the most important information on the most relevant markers.

I realise this change is not ideal and it would be preferable to keep all the figure parts, but it seems as though this would be problematic for the preparation and layout of the proof. Please do let me know if you'd like to discuss this or if you see alternative options on reducing the number of figure panels.

Once the figures are finalised, please convert the files to PDF and ensure that they remain editable (vector format). You can check they are editable by ensuring that no part of them pixelates when

you zoom in and that all text can be selected/highlighted. This requirement is because our in-house illustrators reproduce all figures to conform with Lancet style. We would only need the main-text figures (fig 1-4), as we do not reproduce the supplementary ones.

We have done so.

4. Please include a Data sharing statement in the end matter. It should indicate what data will be shared (eg, the raw data in Appendix 1) and whether additional documents will be shared (eg, you can indicate that the study protocol is in the appendix).

We have done so.

EDITORS' GENERAL POINTS (some of these might not apply to your manuscript; there is no need to respond to these points - please simply go through them and ensure that relevant requirements are satisfied):

- Your revised paper should have fewer than 3500 words (4500 for randomised trials; not including references, COI statements, abstract etc) and a maximum of 30 references (unless it is a systematic review or meta-analysis). The abstract should be structured (background, methods, findings, interpretation, funding) and should be less than 300 words long.
- The main text should be structured as follows: Introduction, Methods, Results, Discussion. Note that subheadings should only be used within the Methods section. For randomised trials, the Methods section should be structured as: Study design and participants, Randomisation and masking, Procedures, Outcomes, Statistical analysis, Role of the funding source (see below).
- If your paper reports results of a randomised trial or a systematic review and meta-analysis, please follow our formatting guidelines for these study types, available to download from <https://www.thelancet.com/for-authors/forms?section=rct> ('RCT guidelines' and 'Systematic reviews and meta-analyses in The Lancet: formatting guidelines').
- Generally, please ensure that all results are presented in a balanced, unbiased way, including clearly stating where findings are non-significant. Note that we often edit manuscripts post-acceptance to ensure the unbiased presentation of results, but it is preferable for authors to ensure this is the case at revision stage.
- The study title should include a study descriptor—eg, randomised trial, case-control study, prospective analysis, population-based study. Titles should be non-declamatory (ie, not state the findings of the paper).
- Please check with your co-authors, and confirm that all names are spelt correctly, and affiliation details for each author are listed correctly (including department, institution, city, state [if applicable], and country). We cannot guarantee that we will be able to correct names and affiliations after publication of your article.
- Please ensure that you include full first names for all authors and please supply (after author names on the title page) one preferred degree per author and indicate in the authorship if any authors are full professors. Note that we can only have one corresponding author,

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- If your author line includes a study group (eg, 'on behalf of the XXXX trial study group'), collaborators' names and affiliations may be listed at the end of the paper or in the appendix. Additionally, if you wish the names of collaborators within a study group to appear on PubMed, please upload with your revision a separate Word document with a list of names of the study group members presented as a two-column table. First and middle names or initials should be placed in the first column, and surnames in the second column. Names should be ordered as you wish them to appear on PubMed. The table will not be included in the paper itself - it's simply used to make sure that PubMed adds the names correctly.
- **References**
References should be in Vancouver style. For references with six authors or fewer, all authors should be listed. For those with seven or more authors, only the first three authors and 'et al' should be listed. Please ensure that reference numbering throughout the manuscript is not inserted with electronic referencing software, such as Endnote, as this is incompatible with our production system (if used, please convert to normal text before resubmission). All web references should have the exact date they were last accessed. With your revised submission please enclose copies of any papers cited as being 'in-press', along with a copy of the acceptance letter from the journal. References that are "submitted" should be removed and citations in the text replaced with "(unpublished data; authors)".
- If accepted, only 5-6 non-text items (figures or tables) can be accommodated in the print edition; additional material can be provided for in an appendix (see below for formatting instructions). Please move items to the appendix, as needed, to adhere to this limit.
- **Figures**
Please supply editable files (eg, EPS files, PowerPoint files, depending on software used to produce them) for all figures such as diagrams and graphs. If figures are composed of photographs or other images, high-resolution files (300 dpi or greater) should be provided. If your figures are annotated, please supply two copies of each of these figures as separate jpgs (one copy annotated and one non-annotated). Our in-house illustrators will annotate according to journal style using the annotated figures as a guide. For multi-part figures, please supply the individual parts as well as a combined version to be used as a guide for our illustrators to recreate the files.
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Images that have been published previously should be accompanied by a statement indicating permission to reproduce the image. If required, further assistance can be obtained from the editorial team. If you have used previously published images, you must obtain and upload permission from the copyright holder, which might be the publisher or the authors. If all the figures are your own and have not been published before, this requirement does not apply.
- Tables should be supplied in a separate Word file (not Excel or fdf/pdf). Each row of data should be in a separate line. Please ensure that rows and columns are not tabbed; data should be entered in cell form.

- It is *Lancet* policy to include a panel in all research papers called "Research in context". Please ensure this panel is included in the manuscript, structured as below (note that no references can be cited in the panel).

Evidence before this study

This section should include a description of all the evidence that the authors considered before undertaking this study. Authors should state: the sources (databases, journal or book reference lists, etc) searched; the criteria used to include or exclude studies (including the exact start and end dates of the search), which should not be limited to English language publications; the search terms used; the quality (risk of bias) of that evidence; and the pooled estimate derived from meta-analysis of the evidence, if appropriate. A summary of what the existing evidence shows should also be included.

Added value of this study

Authors should describe here how their findings add value to the existing evidence (including an updated meta-analysis, if appropriate). IMPORTANT: Please do NOT reiterate the results or describe your study approach (this is already covered by the abstract), but rather explain how the findings extend knowledge in the field and/or address unanswered questions or controversies.

Implications of all the available evidence

Authors should state the implications for practice or policy and future research of their study combined with existing evidence.

- *The Lancet* journals encourage data sharing. If you will share your data please provide an appropriate statement that can be included with the manuscript.

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— <http://www.nature.com/bjc/journal/v93/n4/abs/6602678a.html>

PRISMA - For meta-analyses and systematic reviews

— <http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1000097>

STARD - Diagnostic tests — <http://www.equator-network.org/wp-content/uploads/2015/03/STARD-2015-checklist.pdf>

STROBE - Observational studies — [http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(07\)61602-X/fulltext](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(07)61602-X/fulltext)

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GENERAL REQUIREMENTS:

- *Lancet* style is to provide p values to two significant figures, unless $p < 0.0001$ (note number of decimal places). The exception is certain genetics studies, in which smaller p values can be reported exactly using scientific notation.
- If you have included data for a drug(s), please confirm that the dose, route, and frequency of administration (and the form: eg, a particular salt) are correct.
- Please use rINNs for drug names. For genes and proteins, authors can use their preferred terminology so long as it is in current use by the community, but should provide the

preferred human name from Uniprot (<http://www.uniprot.org/uniprot/>) for proteins and HUGO (<http://www.genenames.org>) for genes at first use to assist non-specialists.

- Where dates are given, please give the exact dates (if known)—ie, day, month, year.
- If appropriate, please use SI units throughout the paper. Note that values for HbA1c should be provided in both % and mmol/mol.
- It is *Lancet* style to give actual numbers (numerator and denominator) together with all percentages, throughout the text and in tables etc.
- Authors often use X-fold when they mean X-times — fold means to the power of ie, 3-fold is to the power of 3 rather than 3 times. Please check and revise if relevant.

SUMMARY (ABSTRACT):

- If your paper reports the results of a trial, your abstract should conform to the CONSORT guidelines for abstracts ([http://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(07\)61835-2/fulltext](http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(07)61835-2/fulltext)), and must include:
 - a. Background: Ending with a sentence indicating the aim of this study.
 - b. Methods: A brief summary of the main patient characteristics (ie, main entry criteria)
 - c. Methods: Details of the regimens used.
 - d. Methods: Details of how randomisation was done (eg, allocation concealment; nature of blinding, if any; how sequence was generated; stratification factors, etc).
 - e. Methods: An explicit description of the actual primary endpoint.
 - f. Methods: The nature by which analyses were done (eg, intention to treat, per protocol).
 - g. Methods: The trial registration number.
 - h. Methods: The status of the trial - is it ongoing/still enrolling/is this an interim analysis, etc?
 - i. Findings: Efficacy data for the primary endpoint only. Secondary outcomes cannot be selectively reported in the abstract, and space restrictions typically prevent all secondary outcomes from being included in the abstract.
 - j. Findings: The most common adverse events (incl actual numbers of patients affected); any serious adverse events.
 - k. Interpretation: please do not just restate your findings. What do they mean, clinically? What are their implications?
 - l. Funding: A line at the end of the abstract stating who funded the research.

MAIN TEXT - METHODS:

- Methods, Study design and participants. If applicable, please ensure that the following items are included:
 - a. Disease setting, including permitted histologies and/or molecular aberrations.
 - b. Age limit for eligibility.

- c. Indicate the means of disease evaluation (eg, RECIST, etc)
- d. Permitted performance status(es) defined.
- e. An indication of estimated survival of eligible patients.
- f. Laboratory tests required to assess eligibility.
- g. Previous treatments permitted/not permitted (and washout period(s)).
- h. If the trial is second-line (or above), mention criteria for determining progression at inclusion.
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- j. Nature of consent sought (eg, written informed consent) and IRB or ethical approval.
- Methods: Randomisation and masking. As applicable, please include details of:
 - a. Sequence generation. A description of the actual method of randomisation (ie, the method used to generate the sequence with which participants are allocated to comparison groups, including details of the methods used to restrict the randomisation—eg, block, stratification). Words and phrases such as "randomised", and "randomly assigned" without qualification are not acceptable.
 - b. Allocation concealment. A description of the method used to conceal assignment of a participant without knowledge of the next assignment in the sequence.
 - c. Implementation. Who generated the allocation sequence, who enrolled participants, and who assigned them to the trial groups
 - d. A description of whether participants, those giving the interventions, those assessing outcomes, and those analysing the data were masked to group assignment; and how was the success of masking evaluated.
- Methods: Procedures. If applicable, please ensure that the following items are included:
 - a. Planned doses of treatment(s).
 - b. Duration of treatment/number of cycles planned.
 - c. Criteria for a patient to be removed from the study.
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- Methods: Outcomes. If applicable, please ensure the following items are included:
 - a. Definition of the primary endpoint.
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Please ensure any post-hoc or exploratory endpoints are clearly described as such.
If your paper is a primary trial report, all prespecified primary and secondary outcomes

specified in the protocol should be listed in the Methods and reported in the Results. If any outcomes prespecified in the protocol are not reported in the present paper, this should be stated in the Outcomes section with a full justification.

- Methods: Statistical analysis. As applicable, please ensure the following items are included:
 - a. Hypothesis, power calculation, and derivation of sample size.
 - b. Definitions of population assessed for primary and secondary outcomes, and for safety (eg, ITT, per protocol, etc).
 - c. Rules for defining patients as not assessable.
 - d. Statistical methods for analysis of the primary and secondary outcomes.
 - e. Any sensitivity analyses, etc.
 - f. Software package (and version number) used for statistical analyses.
 - g. The trial registration number - "This study is registered [as an International Standard Randomised Controlled Trial/with [ClinicalTrials.gov/similar](https://clinicaltrials.gov)], number [ISRCTNxxxxxxx/NCTxxxxxx/similar]."
- Please explain any deviations from the protocol, where appropriate in the Methods section. Please also mention if any protocol amendments affecting trial recruitment or conduct during the study were approved amendments.
- *The Lancet* journals are very supportive of protocol-based research and encourage authors to post the protocol document on a publicly accessible website; a margin link to the website will then be put in the paper. Would you like to do this for your protocol? If so, please provide the link in the Methods section of the main text. Please note that if you do wish to do this, the weblink must be permanent. Alternatively, the protocol can be included in your appendix if you wish - please indicate this in your responses.
- Please give the manufacturer, city, and country for any specific devices mentioned in the Methods section.
- *Lancet* style is to have a 'Role of the funding source' at the end of the methods. The following points need to be addressed in the "Role of the funding source" statement:
 - a. The role of the funders in the study design.
 - b. The role of the funders in the collection, analysis, or interpretation of the data.
 - c. The role of the funders in the writing of the report.
 - d. Those who had access to the raw data (by author initials).
If the funding source had no role then this should be stated (ie, "The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report"). Please also add to this section (if true, or amend if not): "The corresponding author had full access to all of the data and the final responsibility to submit for publication."

MAIN TEXT - RESULTS:

- Results: as applicable, please state the exact dates between which recruitment took place, and/or the study was conducted.

- Results: please explicitly state the number of patients included in analyses, and the number of patients deemed ineligible (and reasons why).
- Results: please give median follow-up (and IQR) for the analyses presented.
- Results: if appropriate, for the main outcome measures, please include a result for each group, plus a point estimate (eg, RR, HR) with a measure of precision (eg, 95% CI) for the absolute difference between groups, in both the abstract Findings section and the main Results section of the paper.
- Please give 95% confidence intervals for hazard ratios/odds ratios.
- Please do not translate HRs/RRs/ORs into percentage increases/decreases, since this can be misleading. Simply indicate the HRs/ORs/RRs and let the reader interpret the data.
- Please add 95% CI to all time-to-event data and other data derived from Kaplan-Meier analyses.
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- Results: safety and tolerability data. Please ensure that the following items are included:
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- If applicable, adverse events should be reported in a table, stratified by grade if appropriate (eg, 1-2, 3, 4 and 5). For graded, adverse events, those of grade 1 or 2 occurring in $\geq 10\%$ of patients should be reported; all grade 3, 4, and 5 events should be reported.

DISCUSSION:

- The Discussion should start with a summary of the main findings of this study. This should not be repeated at the end of the manuscript, as we avoid unnecessary repetition.
- If you have claimed a first, please reword to: "To our knowledge... this is the first time...", since you can never be 100% sure.
- Please ensure that the Discussion contains a section on limitations of the study.

STATEMENTS AND FORMS:

- Author contribution statement
All authors are required to provide a signed author contribution statement form, available to download [here](#) (completed forms should be uploaded with your revised manuscript if not already submitted).
At the end of the manuscript include a "Contributors" section stating the contribution each author has made to the paper. These statements should match exactly those given on the Authors' signed forms. Authors should be referred to by their initials in this section.
Please note, that if an author is added or deleted from the author list, we need a signed

statement from the other authors to agree to the amendment (including the author who was removed).

- Conflict-of-interest statements for individual authors

All authors should complete and return an ICMJE conflict of interest form, available [here](#)

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At the end of the text, under a subheading "Declaration of interest", all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of financial conflicts include employment, consultancies, stock ownership, honoraria, paid expert testimony, patents or patent applications, and travel grants, all within 3 years of beginning the work submitted. If there are no conflicts of interest, authors should state that none exist ("We declare no competing interests."). Authors should be referred to by their initials in this section. The statement should match the information on the supplied ICMJE forms.

- Acknowledgments

The funder(s) of the study should be mentioned at the start of the Acknowledgments section. Please be sure to state whether this study was fully or in part NIH funded, if any authors are employed by NIH, or if any authors are in receipt of an NIH grant. We need written confirmation, including a signature, from everyone who is mentioned in the Acknowledgments section to confirm that they are happy to be quoted in your paper. Signed paper originals are required. The following format can be used:

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Required formatting of data in the appendix:

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- Means should be accompanied by SDs, and medians by interquartile range.
- Exact p values should be provided, unless $p < 0.0001$
- For drug names, recommended international nomenclature (rINN) is required
- References should be in Vancouver style (eg, Smith A, Jones, B, Clements S. Clinical transplantation of tissue-engineered airway. *Lancet* 2008; 372: 1201-09. Hourigan P. Ankle injuries. In: Sports medicine. Chan D, ed. London: Elsevier, 2008: 230-47.) They should be numbered in order of mention in appendix and numbered separately from references in the full paper
- For figures in the appendix, all images must have a minimum resolution of 300 dpi at a width of 107 mm.

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When you submit the revised paper, please provide one "clean" copy and one copy where your changes are tracked. In addition, please provide a separate document listing the reviewers' comments and your replies, point by point. Please also ensure that all elements of the paper and all relevant information has been provided. These documents must be supplied as MS Word files.

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Inflammatory profile of HIV uninfected and co-infected patients with tuberculosis: a prospective cohort study and immunological network analysis

Elsa Du Bruyn, MD^{1,2†} · Kiyoshi F. Fukutani, PhD^{3,4,5†} · Neesha Rockwood, PhD^{1,6,7†} · Charlotte Schutz, MD^{1,2} · Graeme Meintjes, PhD^{1,2} · Maria B. Arriaga, MSc^{3,4} · Juan M. Cubillos-Angulo, MSc^{3,4} · Rafael Tibúrcio, MSc^{3,4} · Alan Sher, PhD⁸ · Catherine Riou, PhD¹ · Katalin A. Wilkinson, PhD^{1,9} · Bruno B. Andrade, MD^{1,3,4,10,11,12‡} · Robert J. Wilkinson, FMedSci^{1,2,6,9‡}

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Abstract

Background

Human immunodeficiency virus type 1 (HIV-1) mediated dysregulation of the immune response to tuberculosis (TB) and its effect on the response to antitubercular treatment (ATT) is incompletely understood. Our aim was to perform an in-depth analysis of the inflammatory profile of HIV-1-uninfected and co-infected TB patients undergoing ATT, with sub-analysis of the effect of antiretroviral therapy and HIV-1 viraemia in the latter group.

Methods

An extensive panel of inflammatory markers were measured in plasma of a prospective patient cohort undergoing ATT at Khayelitsha Site B Clinic, South Africa between March 2013 and July 2014. Plasma samples were collected at baseline (1-5 days after commencing ATT), week 8 and week 20 of ATT. We applied network and multivariate analysis to investigate the dynamic inflammatory profile of these patients in relation to ATT and by HIV status.

Findings

HIV-1 status markedly influenced the inflammatory profile regardless of ATT duration. HIV-1 viral load emerged as a major factor driving differential inflammatory marker expression and impacting on correlation profiles observed in the HIV-1 co-infected group. Unexpectedly, IL-17A emerged as a key correlate of HIV-1 induced inflammation during HIV-TB co-infection. We validated these findings in a second cohort of hospitalized HIV-TB co-infected patients where the number of statistically significant correlations with IL-17A in network analysis predicted mortality.

Interpretation

Our findings demonstrate the effect of HIV-1 co-infection on the complexity of plasma inflammatory profiles in TB patients. Through network analysis we identified IL-17A as an important node in HIV-TB co-infection, thus implicating this cytokine's capacity to correlate with, and regulate, other inflammatory markers. Further mechanistic studies are thus required to identify specific IL-17A related inflammatory pathways mediating immunopathology in HIV-TB co-infection, which may illuminate targets for future host directed therapies.

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Research in context

Evidence before this study

We searched PubMed with the keywords “HIV”, “TB”, “inflammatory markers” and “treatment” for papers published in English since January 2002. We found only 65 articles, with 33 of these being focused on the immune reconstitution inflammatory syndrome and thus not applicable to our study population. The majority of the remaining 32 articles described individual inflammatory markers and their potential to diagnose TB, monitor disease activity or treatment response during HIV-1co-infection. None of these studies used network analysis or any other Systems Immunology approaches.

Added value of this study

A number of previous studies have measured inflammatory marker levels in plasma of HIV-TB co-infected patients, however we show that in depth examination of relationships between inflammatory markers through network analysis can present a valuable tool to advance knowledge of immuno-pathological pathways in complex disease states. This is the first evidence for a central role of IL-17A mediated inflammatory responses in HIV-TB co-infection.

Implications of all the available evidence

By identifying HIV-1 viraemia as the key driver of inflammation during HIV-TB co-infection regardless of duration of ATT, our findings, along with those of others, form a solid scientific basis for advocating early ART initiation in those newly diagnosed with both HIV-1 and TB and diligent monitoring of HIV-1 viral load in TB patients who are established on ART. Furthermore, we identify IL-17A network connectivity as biomarker of mortality in hospitalized patients with HIV-TB co-infection, thus providing grounds for further mechanistic study of this cytokine in HIV-TB.

Introduction

Tuberculosis (TB) remains one of the most deadly infectious diseases, with 1·2 million TB deaths in HIV-1-uninfected individuals and 208,000 HIV-TB co-infected deaths reported in 2019¹. HIV-1 infection is a significant risk factor for TB infection and while anti-retroviral therapy (ART) substantially mitigates TB risk, it remains higher in HIV-1-infected persons on ART compared to HIV-1-uninfected persons².

The influence of HIV-1 co-infection on the immune response to *Mycobacterium tuberculosis* (Mtb) remains poorly understood, as each pathogen compounds the immunopathology associated with the other, adding complexity. Furthermore, the intertwined effects of ART-mediated immune reconstitution and immune clearance of Mtb during ATT makes it difficult to dissect the relative contribution of each to a successful treatment response. There is thus paucity in biomarkers predictive of treatment outcome in HIV-TB co-infection.

We previously addressed this shortfall by investigating expression of the antioxidant enzyme heme oxygenase-1 (HO-1) in the context of HIV-TB co-infection³. There was significantly higher HO-1 expression in HIV-TB co-infected patients compared to those with TB only, with increased expression in the former being driven by HIV-1 viraemia. HO-1 expression decreased with ATT irrespective of HIV-1 status, but not in those who experienced treatment failure or relapse. CRP and ferritin levels also show significant decline during ATT, except in those with HIV-TB co-infection where a slower decline is observed⁴. HIV-TB co-infection induced inflammation appears to take longer than that of TB alone to resolve. This could be explained by high Mtb bacterial load which may occur during advanced immunosuppression, the additive effect of HIV-1 induced inflammation and/or the effect of ART initiation. We previously showed that Mtb load significantly impacts on the inflammatory profile of TB patients⁴⁻⁸. Systemic dissemination of Mtb, more frequently observed in HIV-1 co-infected TB patients, associates with an inflammatory profile distinct from pulmonary TB, and shows delayed response to ATT by network analysis⁸. Thus, network analysis is able to detect important underlying trends in the immune profile of TB cohorts, providing insight into disease pathogenesis. The objective of our study was to employ network analysis to investigate the extent to which the HIV-1 induced immune response contributes to the inflammatory profile in pulmonary TB and the effect of ATT and ART on it.

Materials and Methods

Primary discovery cohort

Participants aged 18 years or older with sputum Xpert MTB/RIF positive rifampin-susceptible pulmonary TB were recruited from the Site B Clinic, Khayelitsha, South Africa, during a prospective cohort study (see Appendix 1 for eligibility criteria, clinical protocol and STROBE statement)^{3,9}. We conducted a post-hoc analysis of plasma inflammatory markers measured at interval after ATT initiation. One participant with HIV-1 coinfection exhibited values below the detection range for > 20% of the analytes and was excluded from the analysis. The University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee (HREC) approved the study (568/2012) and written informed consent was obtained from all study participants.

Validation cohort and control group: clinical characteristics

The validation cohort was part of a larger cohort study¹⁰ that was recruited from 16th January 2014 until 19th October 2016 from patients hospitalized at the Khayelitsha Hospital, Cape Town (see supplementary table 1). HIV-1 infected patients with a CD4 count below 350 cells/ μ L and a high clinical suspicion of new TB were enrolled (see Appendix 1 for eligibility criteria). A control group of HIV-1 infected outpatients with CD4 counts below 350 cells/ μ L and no evidence of TB (asymptomatic, sputum Xpert MTB/RIF negative and no radiographic evidence of TB) were also recruited from the Site B Clinic. All participants provided written informed consent when possible. Those who were eligible, but could not provide informed consent due to decreased level of consciousness were enrolled and followed up daily until they regained the capacity to participate in the informed consent process. UCT HREC approved the study (UCT HREC 057/2013) and per-protocol use of samples and information from those who died before providing informed consent, or who could not provide consent by the end of study follow-up.

Role of Funding Source

The funder of the study played no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and agreed with the the decision to submit for publication.

Procedures

Participants underwent standardized clinical interview and examination after enrolment. Anonymised data was collected from the patient, their clinical folder and laboratory results and captured on a access controlled, secure database. Baseline microbiological testing for Mtb, CD4 count and Viral Load was performed by the National Health Laboratory Services (NHLS). Venous blood samples were collected at enrolment from all participants, at week 8 of ATT from discovery cohort participants, and at week 20 of ATT from those in the discovery cohort who remained sputum culture positive at week 8. Blood samples underwent laboratory processing, plasma was isolated and cryopreserved in -80°C freezers until batched extraction.

Immunoassays

We evaluated a panel of 39 cytokines, acute phase proteins and soluble receptors using different immunoassays in plasma samples. Marker selection was based on their potential role in TB pathogenesis as shown by previous work from our group^{6, 11} and others¹² and included: C-reactive protein (CRP), fibrinogen, ferritin, procalcitonin, serum amyloid protein A (SAA), serum amyloid protein P (SAP), alpha-2-macroglobulin, haptoglobin, tissue plasminogen activator (tPA), matrix metalloproteinase (MMP)-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, TIMP-3, TIMP-4, interleukin (IL)-1 α , IL-1 β , IL-1Ra, IL-6, IL-8, IL-10, IL-12p70, IL-17A, interferon (IFN)- α 2, IFN- γ , tumor necrosis factor (TNF)- α , CCL3, CCL4, CCL11, CXCL10, VEGF, sCD14, and 8-hydroxy-2'-deoxyguanosine (8-OH-dG).

Immunoassay analysis

Median $\pm$ interquartile ranges were used as measures of central tendency and dispersion respectively. The Mann-Whitney *U* test (for two groups) and the Kruskal-Wallis test were used for group and timepoint comparisons.. Fisher's exact test (2x2 comparisons) and Pearson's chi-square (other types of comparisons) were used to compare variables displayed as percentages.

A summary table listing all statistical tests, study timepoints and relevant cohorts used to construct the figures can be found in Appendix 1(supplementary table 2). Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad, La Jolla, CA), STATA 11 (StataCorp, College Station, TX), JMP 14.0 (SAS, Cary, NC) and R 3.1.0 (R Foundation, Vienna, Austria) programs.

Hierarchical cluster analyses (Ward's method), with 100X bootstrap¹³ of log10 transformed and z-score normalized data were employed to depict the expression profile of plasma biomarkers at the various timepoints in the discovery cohort . Dendrograms display the cluster hierarchy represented by Euclidean distances.

Venn diagrams were used to illustrate differentially expressed markers at each study timepoint in the discovery cohort¹⁴.

Fold change (Log10) of plasma markers was calculated where relevant. Differences with p values below 0.05 after Holm-Bonferroni's adjustment for multiple comparisons were considered statistically significant. All comparisons were pre-specified and two-tailed.

The Spearman rank test was used to assess all correlations between all plasma markers and other variables in the discovery cohort.

Network analysis

Profiles of correlations between biomarkers in different subgroups and timepoints were examined using network analysis of bootstrapped Spearman correlation matrices. We displayed correlation matrices as heatmaps and visualized the networks using circular layout network plots using Gephi v. 0.82 with circular layout plug-in. Only correlations with a p-value smaller than 0.05 in at least 80 iterations of 100 bootstraps were included in network visualization. Network density was established calculating the actual connections (known number of connections between nodes) by potential connections (number of potential connections between two nodes; appendix 1; supplementary figure 1). Node analysis was conducted to calculate the number of connections per molecule (the arcs are defined as a significant correlation, $p < 0.05$) by timepoint and build a curve and ANOVA linear trends test to assess variable dependency. The area under the curve (AUC) was used to measure variation between connection numbers over time and identified molecules with most connections.

In the discovery cohort, network analysis was applied to investigate biomarker expression in the overall cohort at different timepoints and to assess the effects of TB and HIV-1 co-infection, ART, and HIV-1 viraemia on biomarker expression.

Network analysis was also used to measure IL-17A network connectivity in the validation and control cohorts. The numbers of connections with IL-17A were compared between: those who died versus survived in the validation cohort; those with a CD4 count below 100 cells/ μ L versus a CD4 count of $\geq$ 100 cells/ μ L in the control cohort; and those with an HIV-1 viral load of $\leq$ 39 RNA copies/mL versus those with a viral load over 39 copies/mL. A receiver operating characteristic (ROC) curve was constructed to evaluate whether the number of connections with IL-17A could predict death in the validation cohort, or low CD4 count or high viral load in the control group. For further explanation of the network analysis procedure, please refer to appendix 1.

Logistic regression and k-fold cross validation models

We used binary logistic regression modelling to identify patient characteristics that may be associated with increased network connectivity/density in the discovery cohort and to test whether IL-17A connectivity was associated with death after adjustment for potential confounding variables in the validation cohort (see supplementary tables 3 and 4; appendix 1).

Results

The discovery cohort consisted of 129 participants (see table 1) of which 76 were HIV-1 coinfectd. Of the HIV-1 infected participants 29 (38.2%) were on ART and 20 (26.3% of HIV-1 infected) of these participants were virally suppressed (HIV VL < 40 copies/mL) at enrolment. . The timepoint of ART initiation was at the discretion of the treating clinician and not standardized, however most participants commenced ART between 2 and 8 weeks after ATT initiation as per national guidelines in South Africa, with 71% (n=54) of the HIV-1 infected cohort being on ART by week 8. The overall age distribution of the HIV-1 infected and uninfected subgroups were similar, however there were more males in the HIV uninfected subgroup and the median body mass index (BMI) of the HIV-1 uninfected subgroup was slightly lower. As expected HIV-1 coinfection was associated with significantly less radiological evidence of “extensive disease” and, their sputum smear was more commonly negative/scanty positive than HIV-1 uninfected participants who tended towards higher smear grades (2+ or 3+). Of all the clinical variables recorded, including male sex, age, weight, smoking, HIV-1 infection, detection of acid-fast bacilli in sputum, presence of cavities and sputum culture conversion at week 8, only HIV-1 infection and the presence of cavities on chest radiograph significantly associated with increased inflammatory network connectivity by binary logistic regression modelling (supplementary table 3).

We examined the expression of inflammatory markers in TB patients before starting ATT (n=129), at 8 weeks (n=129) and at 20 weeks (n=53) of ATT in the discovery cohort . Hierarchical cluster analysis revealed a distinct plasma inflammatory marker expression profile at each of the timepoints (supplementary Fig. 2A). Although the pre-treatment timepoint was characterized by predominantly higher expression of most inflammatory markers accompanied by a decrease over the course of ATT, there were a number of markers that did not follow this pattern. To explore this further we compared the expression of these markers at week 8 and week 20 to the pre-treatment timepoint and identified markers that displayed statistically different levels (Fig. 1A). The majority of the markers showed an early and sustained decrease on ATT.

We used correlation matrices (supplementary Fig. 3) and *circular layout network* plots to visualize differences in correlation profiles between the inflammatory markers and calculated overall network density (Fig.1B and supplementary Fig.2B). Network density was highest pre-treatment and was not significantly different at week 8; however a dramatic decline was observed at week 20 (Fig. 1B).

We conducted node analysis of the top nine markers with the greatest statistically significant correlations per timepoint to define inflammatory markers driving overall network density (Fig. 1C). Considering the number of statistically significant connections for each marker we calculated the AUC for each and ranked the markers in order of their relative contribution to network density. The AUC value thus reflects the overall capacity of the marker to correlate with others in the network over the three study timepoints. The marker with most network connections was IL-17A, which in turn had a significantly higher AUC than all the other ranked markers (supplementary Fig. 2C).

Next we investigated whether HIV-1 co-infection perturbed the plasma inflammatory profile of TB patients. Hierarchical clustering revealed significant differences in inflammatory marker levels between HIV-1-infected (n=76) and -uninfected (n=53) TB patients, which were quantified by calculating fold-change (supplementary Fig. 4). Differences were greatest at the pre-treatment timepoint where six inflammatory markers were significantly lower in HIV-TB co-infected versus HIV-1-uninfected TB patients and seven were significantly higher. When

comparing differentially expressed markers at the pre-treatment timepoint versus week 8 and week 20 on treatment respectively, the majority were decreased in both HIV-1-infected and -uninfected TB patients (Fig. 2A).

Upon assessing significant correlations of plasma inflammatory markers in the HIV-1 infected subgroup with either CD4 count or HIV-1 viral load we found most markers to show significant negative correlation with CD4 count and significant positive correlation with HIV-1 viral load (Fig. 2B).

Considering the possibility that HIV-1 co-infection may have a substantial effect on individual plasma inflammatory marker expression in TB patients, we investigated how this influenced correlations between these markers. Correlation matrices of HIV-1 co-infected versus -uninfected participants provided evidence of differential correlation between the inflammatory markers in each of these subgroups and timepoints (supplementary Fig. 5 and supplementary Fig. 6). Network analysis revealed significantly greater density in the HIV-1 co-infected compared to the HIV-1-uninfected group pre-treatment and at week 8, with no significant difference being observed at week 20 (supplementary Fig. 5A and B). A linear decrease in network density was observed in HIV-1 co-infected participants, whereas HIV-1-uninfected participants showed slightly increased network density compared to pretreatment at week 8, with significant decrease only occurring at week 20. The top 3 markers contributing most to overall network density by AUC in the HIV-1-uninfected subgroup were CXCL10, CRP and IFN- α 2, whereas the top 3 markers in the HIV-1 co-infected subgroup were IL-17A, IL-1 β and TNF- α (supplementary Fig 5C and D). IL-17A was the most, and TNF- α the third most influential markers in network connectivity analysis of the cohort as a whole.

To further investigate the effect of HIV-1 on the plasma inflammatory profile we assessed the impact of ART on network connectivity in HIV-TB co-infection. Most participants in the ART established group were virally suppressed with 20/29 (68.9%) and a trend towards higher median CD4 T cell count than that of the ART naïve subgroup (supplementary Fig. 7B). Although ART status influenced the expression of some of the inflammatory markers in HIV-TB co-infected participants at various timepoints there was no statistically significant difference between the ART established and naïve subgroups in terms of frequency of extensive disease on chest radiograph, culture positivity at week 8 and unfavorable outcomes (supplementary Fig. 7A and B).

Despite only modest differences in individual plasma marker expression being observed between ART naïve and established co-infected participants (supplementary Fig. 7A), network analysis of correlation matrices (supplementary Fig. 8) for each timepoint pre- and post-ATT showed marked differences (supplementary Fig. 9A). Pre-treatment network density was significantly higher in ART naïve participants compared to those established on ART (supplementary Fig. 9B). Strikingly, similar network density to that of the pre-treatment timepoint was observed in the ART naïve group despite eight weeks of ATT, contrasting with the ART established group at week 8 who displayed significantly decreased network density. At week 20 both ART naïve and established groups displayed similarly low network density. Nodal analysis revealed that IL-17A again demonstrated the greatest nodal connectivity in ART naïve co-infected participants, with IL-1 β and TNF- α in second and third place respectively (supplementary Fig. 9C). In the ART-established group IFN- α 2, IL-17A and IL-1 β were the markers with greatest nodal connectivity (supplementary Fig. 9D).

Not all ART established participants were virally suppressed and we hypothesized that HIV-1 viraemia contributed to their observed inflammatory profile. Upon stratifying HIV-1-infected participants by those who had suppressed versus unsuppressed viral loads and employing inflammatory network analysis of correlation matrices (supplementary Fig. 10), we found that this was indeed the case (supplementary Fig. 11 and Fig. 3A). Those who had suppressed HIV-1 viral loads had comparable low network density at all timepoints, including pre-treatment. Furthermore, those who were viraemic exhibited high network density pre-treatment, no statistically significant change in density at week 8 with significant decrease in network density only occurring at week 20. Nodal analysis again identified IL-17A as the most connected node in those with unsuppressed HIV-1 viral loads (Fig. 3B and C).

Finally, we applied network analysis to two validation studies: one to investigate mortality in hospitalized HIV-TB co-infected patients and the other examining HIV progression in the absence of active TB (see supplementary table 1). The hospitalized HIV-1 co-infected cohort¹⁰ consisted of 516 patients admitted with TB, of which 113 died within 12 weeks of enrolment. There were 29 participants in the outpatient control group who were HIV-1 infected without evidence of TB. Those of the hospitalized group who died tended to be slightly older than those who survived and those in the outpatient control group. There were 272 females (52.7%) and 244 males (47.3%) in the hospitalized cohort, with similar sex distribution by subgroup. The outpatient control group were predominantly female (72.4%). Those of the hospitalized cohort who died tended to have lower median CD4

counts than those who survived and the outpatient controls. The majority of the hospitalized cohort was not on ART with only 186 (36%) being on ART at enrolment. ART data is not available for the outpatient control group.

In the hospitalized co-infected cohort¹⁰ we found the number of connections with IL-17A to be significantly higher in those who died (n=113) compared to those who survived (n=394) (Fig. 4). Binary logistic regression modelling confirmed that this association was not confounded by the effect of microbiologic confirmation of TB, weight, male sex or age (supplementary table 4). The number of connections with IL-17A through network analysis exhibited 88.0% sensitivity and 89.0% specificity to differentiate those who died versus survived hospital admission for HIV-1-associated TB. In the HIV-1 infected outpatient control group without active TB the number of nodal connections with IL-17A was significantly higher in those with lower CD4 counts (supplementary Fig. 12A) and unsuppressed viral loads (supplementary Fig. 12B). Conversely, network analysis of the primary discovery cohort showed that TB infection in the absence of HIV-1 co-infection contributed little to IL-17A network connectivity, even in those with extensive TB disease on chest radiograph (supplementary Fig. 13).

Discussion

We performed detailed analysis to better understand the effect of HIV-1 co-infection on the inflammatory profile of a well characterized cohort of TB patients undergoing ATT. We did not observe any decline in the plasma network density between pre-treatment and week 8 of ATT, significant decline in density was only observed at week 20 in the discovery cohort. This could not be explained by delayed culture conversion, a factor known to significantly influence the plasma inflammatory profile⁴⁻⁸, as the majority of participants were no longer sputum culture positive at week 8. Importantly, stratification by HIV status revealed that network density in the cohort as a whole was driven by that of the HIV-1 coinfecting subgroup, owing to much greater network density observed at week 8 than that of HIV-1 uninfected TB patients. When we further stratified the HIV-1 coinfecting subgroup by ART status we found that the ART naïve inflammatory profile seemed to underlie the high network density we had observed in the the greater HIV-1 coinfecting group, thus potentially offering explanation for the delayed decline in network density in the cohort as a whole. The majority of the HIV-1 infected group were not on ART at enrolment (61.8%), with 29% yet to commence ART by week 8. Thus, in absence of availability of viral load data at week 8, and considering that many HIV-1 infected participants were newly commenced on ART or still ART naïve at this timepoint, it can be assumed that a sizable proportion of the HIV-1 coinfecting group would still have been viraemic at week 8. Dissecting this effect further we showed that HIV-1 viraemia was driving network connectivity in HIV-TB co-infected patients and that the overall immune profile of those who were virally suppressed exhibited minimal network density. Through network analysis of key inflammatory mediators we have thus found that HIV-1 viraemia during HIV-TB coinfection mediates ongoing systemic inflammatory activity, with ART mediated viral suppression abrogating this effect.

Little is known about the individual contribution of HIV-1 and TB to systemic immune activation and inflammation observed during co-infection. *In vitro* models of macrophage co-infection suggest that Mtb-derived gene expression predominates over that of HIV-1, with particular upregulation of pro-inflammatory pathways and type I interferon (IFN) gene signalling¹⁵. Conversely, productive infection of monocyte-derived macrophages with HIV-1 only did not activate inflammatory pathways and was not associated with interferon gene expression¹⁶, which was later shown to be part of an HIV-1 mediated immune evasion strategy¹⁷. However, our *ex vivo* findings indicate that HIV-1 viraemia may play a greater role in promoting inflammation in HIV-TB co-infection than previously appreciated.

Another important hypothesis generating finding was that IL-17A was the most important node during network analysis in the cohort as a whole, as well as in the ART naïve and HIV-1 virally unsuppressed subgroups, but did not feature in the ranking of the top influential nodes in HIV uninfected TB patients. This implicates IL-17A and its related immune networks in the inflammatory response to HIV-TB, especially in the setting of unsuppressed HIV-1 viral load. By applying network analysis to the hospitalized HIV-TB coinfecting cohort we found that the overall number of connections with IL-17A differentiated those who died and survived hospital admission with high sensitivity and specificity. We show that in an HIV-1 infected outpatient control group without TB, advanced HIV-1 as denoted by low CD4 counts (CD4 count < 100 cells/mL) and high viral loads (VL > 39 copies/mL) was associated with higher IL-17A network connectivity, similar to those who died of HIV-TB. By contrast, evidence of severe TB disease on chest radiograph was not associated with markedly increased IL-17A network connectivity. IL-17A network connectivity thus serves as a read-out of higher systemic inflammation and immune activation induced by advanced HIV-1 infection and uncontrolled HIV-1 viral replication and denotes higher mortality risk in HIV-TB co-infection.

IL-17A is produced by various cell types with widespread expression of its receptor being observed and its pluripotent effects ranging from mucosal barrier protection to immunopathology in autoimmune disorders^{18,19}. IL-17 may play a beneficial role in TB by contributing to granuloma maturity, however it can also induce tissue damage through induction of MMPs²⁰⁻²². Most studies report higher IL-17 production in latent TB infection than in active TB²³, thus there is minimal existing evidence of the induction IL-17 related cytokine networks by Mtb per se. Indeed, our findings indicate that increased IL-17A network connectivity is mediated by HIV-1 viraemia and not TB. HIV-1-Tat protein induces transcriptional dysregulation with particular activation of IL-17 signalling pathways²⁴. Profound depletion of IL-17 producing Th17 cells, are observed at mucosal barriers early after HIV-1 infection, leading to chronic immune activation²⁵. The combination of ongoing mucosal inflammation, HIV-1 replication and Mtb co-infection may result in heightened immune activation leading to constant triggering of the remaining IL-17A producing cell populations during HIV-TB co-infection. This is exemplified by findings of upregulated Th17 polarizing cytokines and transcription factors in pleural fluid of HIV-TB co-infected patients with TB pleurisy, despite numeric depletion of Th17 cells²⁶. Indeed it has been shown that IL-17A synergises with other cytokines, especially TNF- α and IL-1 β to mediate induction of IL-6 and IL-8 and promotes the synthesis of pro-inflammatory cytokines such as TNF- α and IL-1 β ^{27, 28,29}. IL-17A thus displays pleiotropic capacity to influence cytokine signalling and can activate diverse signalling pathways¹⁸. These immunological relationships coordinated by IL-17A are reflected through our network analysis findings, with IL-17A, IL-1 β and TNF- α featuring as the top 3 most influential nodes in our HIV-1-co-infected and ART naïve groups. This is reproduced in the top four most influential nodes of HIV-1-infected viremic participants, with IL-8 showing the second most numerous network connectivity after IL-17A, followed by IL-1 β and TNF- α .

Our study is limited by the fact that we do not know the approximate duration of HIV-1 infection of participants, a factor that could affect the inflammatory profile. Furthermore, the baseline median CD4 count of the discovery cohort was 192 cells/ μ L, thus it is difficult to anticipate to what extent our findings may be applicable to those with higher CD4 counts and the timing of ART initiation varied in the ART naïve, HIV-1 infected participants precluding analysis to assess for the effect of ART duration. Only those who were Mtb culture positive donated plasma at week 20, representing a bias inherent to the study design. We did not use laboratory testing to exclude all possible other concomitant opportunistic infections, but the majority of these would have been detected by clinical review. We measured only soluble inflammatory markers, however further investigation of our findings employing different laboratory technologies such as flow cytometry or transcriptome analysis could provide further insight.

A comprehensive assessment of inflammatory responses in the immune pathogenesis of HIV-TB coinfection remains challenging owing to numerous potential biomarkers and complex interactions. Here we add to mounting evidence that network analysis represents a useful, rigorous statistical approach allowing biomarker discovery and identification of immune pathways associated with disease severity and treatment outcome. While IL-17A related inflammatory networks have been extensively studied in autoimmune disease, here we show for the first time its integral role in HIV-TB co-infection, concurring with suggestions that autoimmunity may represent an overlooked component of the pathological immune response to TB³⁰. Further studies are required to confirm whether our finding of upregulated IL-17A related inflammatory networks in HIV-TB are indeed associated with an auto-immune response to TB, to elucidate the exact mechanism whereby IL-17A network connectivity is enhanced during HIV-TB co-infection and how HIV-1 viraemia drives this effect. These mechanistic studies might reveal whether IL-17A related inflammatory pathways are cause or consequence of detrimental outcomes in HIV-1 and HIV-TB infection and thus potentially inform novel therapeutic targets.

Conflict of Interest Statement

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. RJW reports grants from Wellcome, grants from National Institutes of Health, grants from European and Developing Countries Clinical Trials Partnership, grants from Cancer Research UK, grants from UK Research and innovation, during the conduct of the study.

Data Sharing Statement

Detailed description of network analysis methodology as well as the full clinical protocol is available in appendix 1. All raw Luminex immunoassay data is available in appendix 2.

Authors and Contributors

EDB, NR, CS, KAW, BBA performed experiments; EDB, NR, KAW, CS, GM, CR, RJW, BBA designed experiments; EDB, NR, KFF, CR, BBA analyzed the data; NR and CS conducted the clinical recruitment, administered the projects and curated the respective datasets; KAW, GM, RJW provided materials and infrastructural support; EDB, KFF, NR, JMC-A, MBA, RT, CR, KAW, CS, GM, AS, RJW and BBA wrote the manuscript.

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Table 1. Characteristics of study participants.

Study group N	All 129	HIV-1 infected 76	HIV-1 uninfected 53	p-value
Male	74 (57.4)	33 (43.4)	41 (77.4)	<0.001
Age (years)	35.1 (30–43.7)	35.6 (30.6–42.1)	34.9 (27.7–50.7)	0.969
BMI (kg/m²)	21 (19–23)	22 (20–24)	20 (19–23)	0.037
Diabetes mellitus	10 (7.75)	5 (6.5)	5 (9.4)	0.740
Smoking history	60 (43.5)	29 (38.2)	31 (58.5)	0.001
AFB smear				0.007
Negative/scanty	47 (36.4)	36 (47.4)	11 (16.9)	
1+	23 (17.8)	15 (19.7)	8 (15.1)	
2+	27 (20.9)	12 (15.8)	15 (28.3)	
3+	32 (24.8)	13 (17.1)	19 (35.8)	
Extensive radiological disease	91 (70.5)	42 (55.3)	49 (92.5)	<0.001
Cavitation	67 (51.5)	34 (44.7)	33 (62.3)	0.075
Culture conversion at week eight	75 (58.1)	50 (65.8)	25 (47.2)	0.054
Death	5 (3.8)	5 (6.6)	0	0.057
Baseline CD4 T (cells/μl)	-	192 (67.5-365)	-	-
Baseline viral load (copies/ml)	-	108692 (126-385633)	-	-
Number on ART	-	29 (38.2)	-	-
Number virally suppressed (viral load < 40 copies/ml)		20 (26.3)	-	-

Data above reflect the number (%) except for age, BMI, CD4 and Viral load where the median value (interquartile range) is displayed. BMI: Body Mass Index. AFB: acid-fast bacilli

Figures

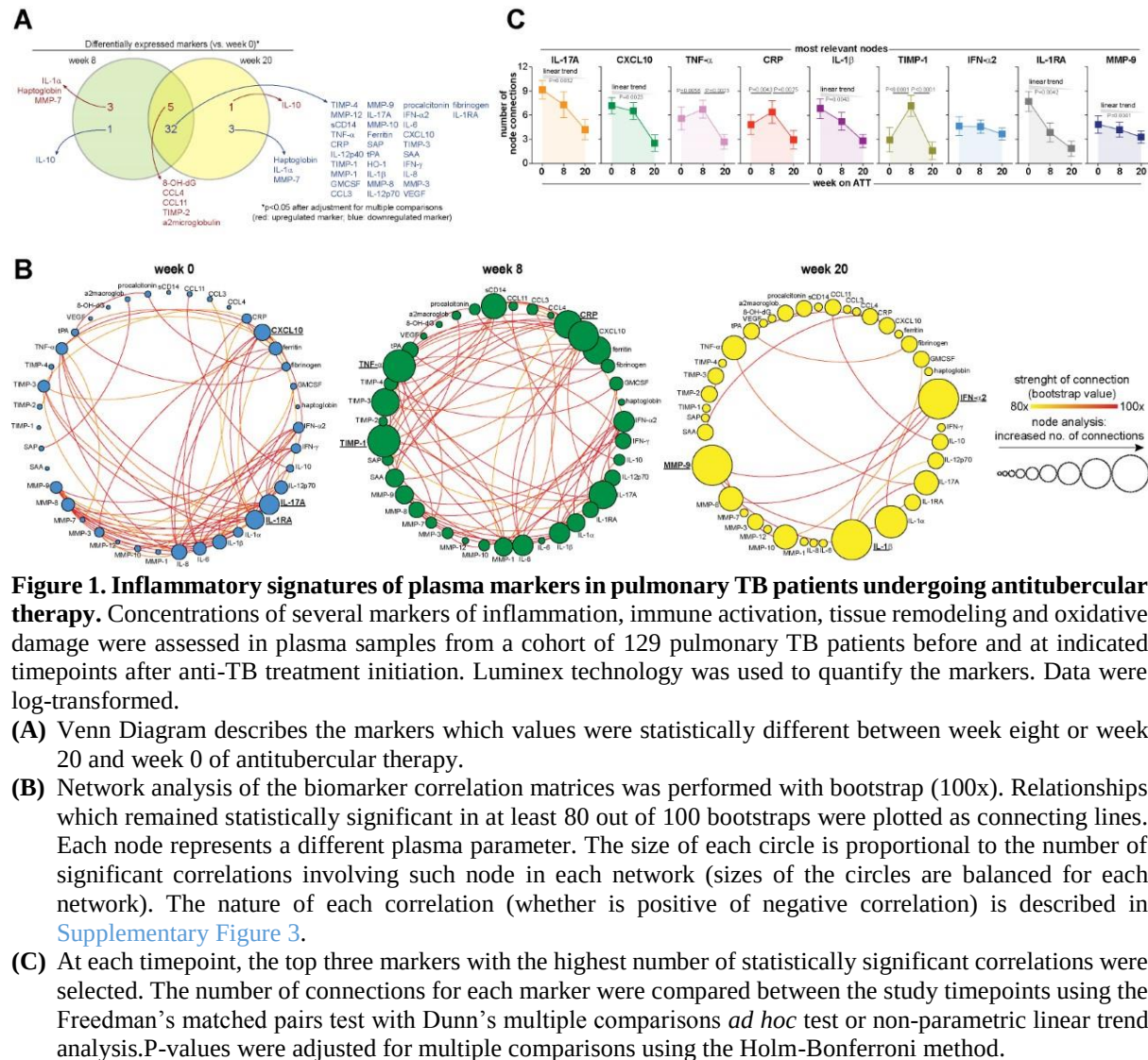


Figure 1. Inflammatory signatures of plasma markers in pulmonary TB patients undergoing antitubercular therapy. Concentrations of several markers of inflammation, immune activation, tissue remodeling and oxidative damage were assessed in plasma samples from a cohort of 129 pulmonary TB patients before and at indicated timepoints after anti-TB treatment initiation. Luminex technology was used to quantify the markers. Data were log-transformed.

- (A) Venn Diagram describes the markers which values were statistically different between week eight or week 20 and week 0 of antitubercular therapy.
- (B) Network analysis of the biomarker correlation matrices was performed with bootstrap (100x). Relationships which remained statistically significant in at least 80 out of 100 bootstraps were plotted as connecting lines. Each node represents a different plasma parameter. The size of each circle is proportional to the number of significant correlations involving such node in each network (sizes of the circles are balanced for each network). The nature of each correlation (whether is positive or negative correlation) is described in [Supplementary Figure 3](#).
- (C) At each timepoint, the top three markers with the highest number of statistically significant correlations were selected. The number of connections for each marker were compared between the study timepoints using the Freedman's matched pairs test with Dunn's multiple comparisons *ad hoc* test or non-parametric linear trend analysis. P-values were adjusted for multiple comparisons using the Holm-Bonferroni method.

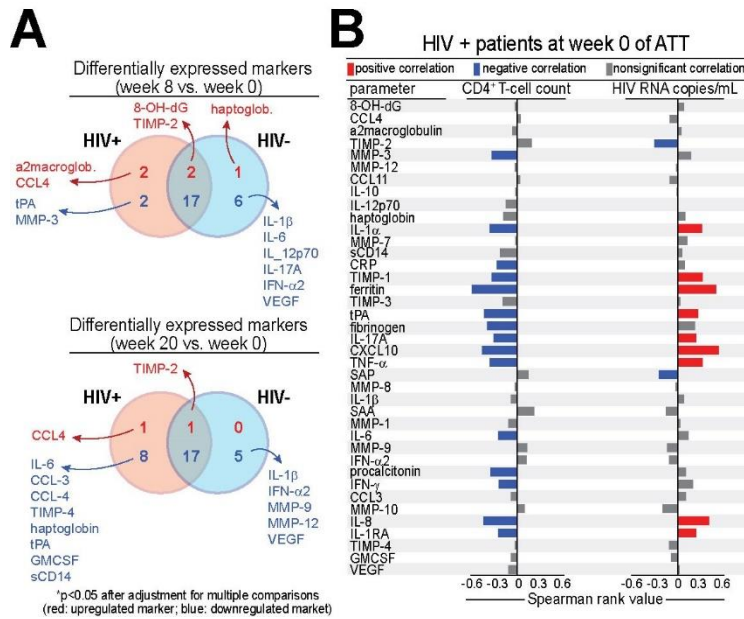


Figure 2. Differential expression of plasma markers in pulmonary TB patients stratified according to HIV-1 infection status.

(A) Venn Diagrams describe the markers whose values were statistically different between week eight or week 20 and week 0 of antitubercular therapy in subgroups of patients stratified according to HIV-1 infection status

(B) Plasma levels of the indicated markers measured at pre-ATT were tested for correlations with CD4⁺ T cell counts as well as HIV-1 RNA viral loads using the Spearman correlation rank test.

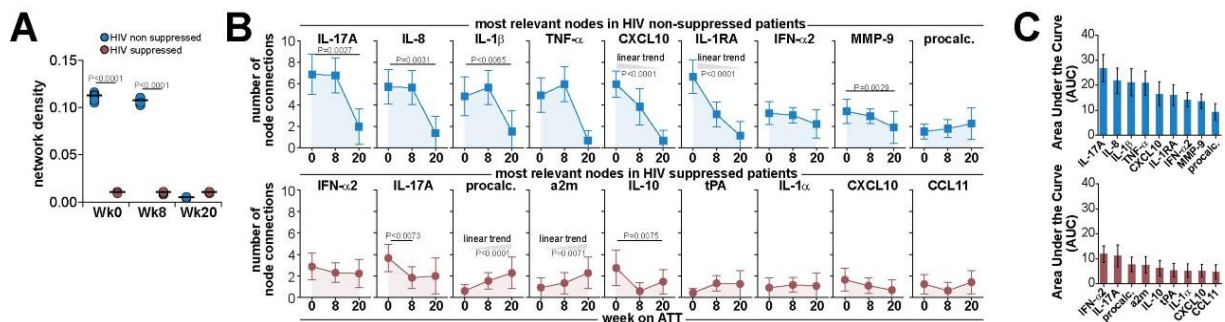


Figure 3. Inflammatory signatures of plasma markers in pulmonary HIV-1-TB co-infected patients stratified by HIV-1 viral suppression during ATT.

(A) Network densities of each bootstrap were calculated for each study group and timepoint as described in Materials and Methods. Data were compared using the Kruskal-Wallis test with Dunn's multiple comparisons *ad hoc* test.

(B) At each timepoint, the top three markers with the highest number of statistically significant correlations were selected. The number of connections for each marker were compared between the study timepoints using the Freedman's matched pairs test with Dunn's multiple comparisons *ad hoc* test or non-parametric linear trend analysis. P-values were adjusted for multiple comparisons using the Holm-Bonferroni method.

(C) Area under the curves (AUC) values were calculated for each marker shown in (C).

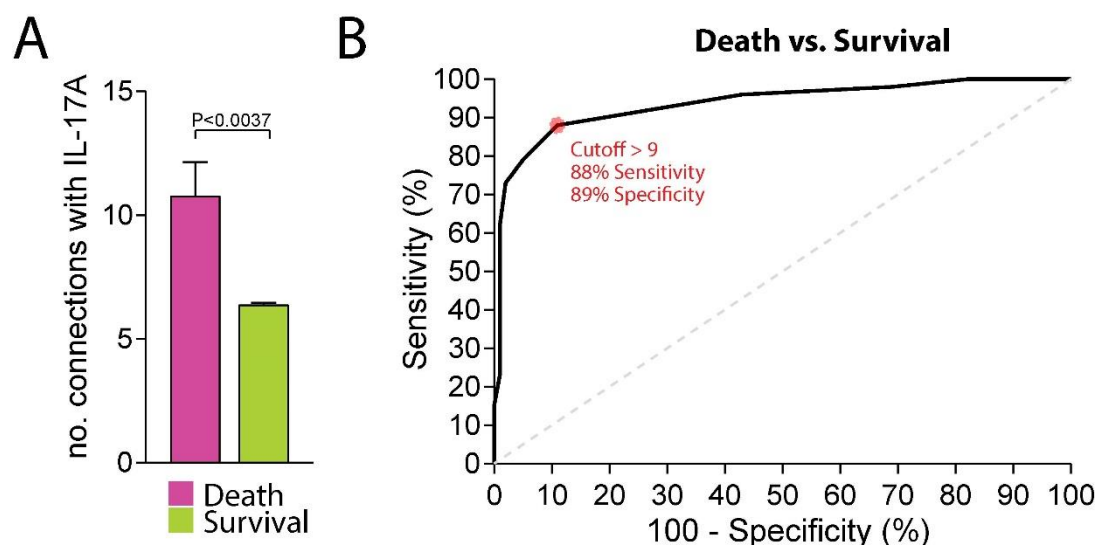


Figure 4. Inflammatory signatures of plasma markers in hospitalized pulmonary HIV-1-TB patients undergoing ART at week 0 of antitubercular therapy.

Number of connections involving IL-17A were compared in a prospective study which investigated determinants of mortality in hospitalized patients with HIV-1-associated TB.

References

1. WHO. Global Tuberculosis Report. 2020. < <https://www.who.int/tb/global-report-2020> >, (Accessed 18/01/2021).
2. Gupta A, Wood R, Kaplan R, Bekker LG, Lawn SD. Tuberculosis incidence rates during 8 years of follow-up of an antiretroviral treatment cohort in South Africa: comparison with rates in the community. *PLoS One*. 2012;**7**(3):e34156.
3. Rockwood N, Costa DL, Amaral EP, et al. *Mycobacterium tuberculosis* Induction of Heme Oxygenase-1 Expression Is Dependent on Oxidative Stress and Reflects Treatment Outcomes. *Front Immunol*. 2017;**8**:542.
4. Miranda P, Gil-Santana L, Oliveira MG, et al. Sustained elevated levels of C-reactive protein and ferritin in pulmonary tuberculosis patients remaining culture positive upon treatment initiation. *PLoS One*. 2017;**12**(4):e0175278.
5. Mesquita ED, Gil-Santana L, Ramalho D, et al. Associations between systemic inflammation, mycobacterial loads in sputum and radiological improvement after treatment initiation in pulmonary TB patients from Brazil: a prospective cohort study. *BMC Infect Dis*. 2016;**16**:368.
6. Andrade BB, Pavan Kumar N, Amaral EP, et al. Heme Oxygenase-1 Regulation of Matrix Metalloproteinase-1 Expression Underlies Distinct Disease Profiles in Tuberculosis. *J Immunol*. 2015;**195**(6):2763-73.
7. Oliveira-de-Souza D, Vinhaes CL, Arriaga MB, et al. Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations. *Sci Rep*. 2019;**9**(1):8002.
8. Vinhaes CL, Oliveira-de-Souza D, Silveira-Mattos PS, et al. Changes in inflammatory protein and lipid mediator profiles persist after antitubercular treatment of pulmonary and extrapulmonary tuberculosis: A prospective cohort study. *Cytokine*. 2019;**123**:154759.
9. Rockwood N, Sirgel F, Streicher E, Warren R, Meintjes G, Wilkinson RJ. Low Frequency of Acquired Isoniazid and Rifampicin Resistance in Rifampicin-Susceptible Pulmonary Tuberculosis in a Setting of High HIV-1 Infection and Tuberculosis Coprevalence. *J Infect Dis*. 2017;**216**(6):632-40.
10. Schutz C, Barr D, Andrade BB, et al. Clinical, microbiologic, and immunologic determinants of mortality in hospitalized patients with HIV-associated tuberculosis: A prospective cohort study. *PLoS Med*. 2019;**16**(7):e1002840.
11. Mayer-Barber KD, Andrade BB, Oland SD, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature*. 2014;**511**(7507):99-103.
12. Ernst JD. The immunological life cycle of tuberculosis. *Nat Rev Immunol*. 2012;**12**(8):581-91.
13. Friedman N, Goldszmidt M, Wyner A. Data analysis with bayesian networks: a bootstrap approach. *Proceedings of the Fifteenth conference on Uncertainty in artificial intelligence*; Stockholm, Sweden. 2073819: Morgan Kaufmann Publishers Inc.; 1999. p. 196-205.
14. Prada-Medina CA, Fukutani KF, Pavan Kumar N, et al. Systems Immunology of Diabetes-Tuberculosis Comorbidity Reveals Signatures of Disease Complications. *Sci Rep*. 2017;**7**(1):1999.
15. Tsang J, Chain BM, Miller RF, et al. HIV-1 infection of macrophages is dependent on evasion of innate immune cellular activation. *AIDS*. 2009;**23**(17):2255-63.
16. Maddocks S, Scandurra GM, Nourse C, et al. Gene expression in HIV-1/*Mycobacterium tuberculosis* co-infected macrophages is dominated by *M. tuberculosis*. *Tuberculosis (Edinb)*. 2009;**89**(4):285-93.
17. Rasaiyaah J, Tan CP, Fletcher AJ, et al. HIV-1 evades innate immune recognition through specific cofactor recruitment. *Nature*. 2013;**503**(7476):402-5.
18. McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 Family of Cytokines in Health and Disease. *Immunity*. 2019;**50**(4):892-906.
19. Veldhoen M. Interleukin 17 is a chief orchestrator of immunity. *Nat Immunol*. 2017;**18**(6):612-21.
20. Okamoto Yoshida Y, Umemura M, Yahagi A, et al. Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. *J Immunol*. 2010;**184**(8):4414-22.
21. Freches D, Korf H, Denis O, Havaux X, Huygen K, Romano M. Mice genetically inactivated in interleukin-17A receptor are defective in long-term control of *Mycobacterium tuberculosis* infection. *Immunology*. 2013;**140**(2):220-31.
22. Singh S, Maniakis-Grivas G, Singh UK, et al. Interleukin-17 regulates matrix metalloproteinase activity in human pulmonary tuberculosis. *J Pathol*. 2018;**244**(3):311-22.
23. Jasenosky LD, Scriba TJ, Hanekom WA, Goldfeld AE. T cells and adaptive immunity to *Mycobacterium tuberculosis* in humans. *Immunol Rev*. 2015;**264**(1):74-87.
24. Johnson TP, Patel K, Johnson KR, et al. Induction of IL-17 and nonclassical T-cell activation by HIV-Tat protein. *Proc Natl Acad Sci U S A*. 2013;**110**(33):13588-93.
25. Veazey RS. Intestinal CD4 Depletion in HIV / SIV Infection. *Curr Immunol Rev*. 2019;**15**(1):76-91.

26. Korb VC, Phulukdaree A, Lalloo UG, Chuturgoon AA, Moodley D. TB/HIV pleurisy reduces Th17 lymphocyte proportion independent of the cytokine microenvironment. *Tuberculosis (Edinb)*. 2016;99:92-9.
27. Noack M, Beringer A, Miossec P. Additive or Synergistic Interactions Between IL-17A or IL-17F and TNF or IL-1beta Depend on the Cell Type. *Front Immunol*. 2019;10:1726.
28. Katz Y, Nativ O, Beer Y. Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a "fine-tuning cytokine" in inflammation processes. *Arthritis Rheum*. 2001;44(9):2176-84.
29. Jovanovic DV, Di Battista JA, Martel-Pelletier J, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol*. 1998;160(7):3513-21.
30. Elkington P, Tebruegge M, Mansour S. Tuberculosis: An Infection-Initiated Autoimmune Disease? *Trends Immunol*. 2016;37(12):815-8.

Inflammatory profile of HIV uninfected and co-infected patients with tuberculosis: a prospective cohort study and immunological network analysis

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Abstract

Background

Human immunodeficiency virus type 1 (HIV-1) mediated dysregulation of the immune response to tuberculosis (TB) and its effect on the response to antitubercular treatment (ATT) is incompletely understood. Our aim was to perform an in-depth analysis of the inflammatory profile of HIV-1-uninfected and co-infected TB patients undergoing ATT, with sub-analysis of the effect of antiretroviral therapy and HIV-1 viraemia in the latter group.

Methods

We performed a prospective cohort study of patients with confirmed drug sensitive tuberculosis undergoing treatment at the Khayelitsha Site B Clinic, South Africa. An extensive panel of inflammatory markers, cytokines, acute phase proteins and soluble receptors were measured in plasma of a prospective cohort undergoing ATT at Khayelitsha Site B Clinic, South Africa between March 2013 and July 2014. Plasma samples were collected at baseline (1-5 days after commencing ATT), week 8 and week 20 of ATT. We applied basic and advanced statistical analysis, including network and multivariate analysis, to investigate the dynamic inflammatory profile of these patients in relation to ATT and by considering the impact of HIV co-infection status. We validated our findings on a second prospective cohort of patients hospitalized at Khayelitsha Hospital, South Africa with HIV-TB co-infection. For the purpose of further validation of the HIV-1 specific influence on the inflammatory profile we recruited a cross-sectional control group of patients with HIV-1 infection, no concomitant active TB and relatively low CD4 counts (≤ 350 cells/ μ l) at the Site B Clinic.

Findings

Patients were recruited to the primary discovery cohort between 1st March 2013 and 31st July 2014, and followed up over the course of their ATT. Plasma samples were collected at baseline (1-5 days after commencing ATT), week 8 and week 20 of ATT. We found that HIV-1 status markedly influenced the inflammatory profile regardless of ATT duration. HIV-1 viral load emerged as a major factor driving differential inflammatory marker expression and impacting on correlation profiles observed in the HIV-1 co-infected group. Unexpectedly, IL-17A emerged as a key correlate of HIV-1 induced inflammation during HIV-TB co-infection. We validated these findings in a second cohort of hospitalized HIV-TB co-infected patients where the number of statistically significant correlations with IL-17A this cytokine in network analysis predicted mortality.

Interpretation

Our findings demonstrate the effect of HIV-1 co-infection on the complexity of plasma inflammatory profiles in TB patients. Through network analysis we identified IL-17A as an important node in HIV-TB co-infection, thus implicating this cytokine's capacity to correlate with, and regulate, other inflammatory markers. Further mechanistic studies are thus required to identify specific IL-17A related inflammatory pathways mediating immunopathology in HIV-TB co-infection, which may illuminate targets for future host directed therapies.

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South African Medical Research Council

Research in context

Evidence before this study

We searched PubMed with the keywords "HIV", "TB", "inflammatory markers" and "treatment" for papers published in English since January 2002. We found only 65 articles, with 33 of these being focused on the immune reconstitution inflammatory syndrome and thus not applicable to our study population. The majority of the remaining 32 articles described individual inflammatory markers and their potential to diagnose TB, monitor disease activity or treatment response during HIV-1 co-infection. None of these studies used network analysis or any other Systems Immunology approaches.

Added value of this study

A number of previous studies have measured inflammatory marker levels in plasma of HIV-TB co-infected patients, however we show that in depth examination of relationships between inflammatory markers through network analysis can present a valuable tool to advance knowledge of immuno-pathological pathways in complex disease states. This is the first evidence for a central role of IL-17A mediated inflammatory responses in HIV-TB co-infection.

94 **Implications of all the available evidence**
95 By identifying HIV-1 viraemia as the key driver of inflammation during HIV-TB co-infection regardless of
96 duration of ATT, our findings, along with those of others, form a solid scientific basis for advocating early ART
97 initiation in those newly diagnosed with both HIV-1 and TB and diligent monitoring of HIV-1 viral load in TB
98 patients who are established on ART. Furthermore, we identify IL-17A network connectivity as biomarker of
99 mortality in hospitalized patients with HIV-TB co-infection, thus providing grounds for further mechanistic
100 study of this cytokine in HIV-TB.

Introduction

Tuberculosis (TB) remains one of the most deadly causes more deaths than any other infectious diseases, with 1.2 million TB deaths in HIV-1-uninfected individuals and 208,000 251,000 HIV-TB co-infected deaths reported in 2018²⁰¹⁹¹. People living with HIV-1 infection is a significant risk factor for are at increased risk of TB infection and while and accounts for 8-6% of TB cases worldwide. Anti-retroviral therapy (ART) substantially mitigates TB risk, it, however TB risk remains higher in HIV-1-infected persons on ART compared to than those who are HIV-1-uninfected persons².

The influence of HIV-1 co-infection on the immune response to *Mycobacterium tuberculosis* (Mtb) remains poorly understood, as each pathogen compounds the immunopathology associated with the other, adding complexity. Furthermore, the intertwined effects of ART-mediated immune reconstitution and immune clearance of Mtb during ATT makes it difficult to dissect the relative contribution of each to a successful treatment response. There is thus paucity in biomarkers predictive of treatment outcome in HIV-TB co-infection.

We previously addressed this shortfall by in a study investigating expression of the antioxidant enzyme heme oxygenase-1 (HO-1) in the context of HIV-TB co-infection³. There was significantly higher HO-1 expression in HIV-TB co-infected patients compared to those with TB only, with increased expression in the former being driven by HIV-1 viraemia. Furthermore, HO-1 expression decreased with ATT irrespective of HIV-1 status, but not in those who experienced treatment failure or relapse. CRP and ferritin levels also show statistically significant decline during ATT, except in those with HIV-TB co-infection where a much slower decline is observed⁴. It appears that the inflammation induced by HIV-TB co-infection induced inflammation appears to takes longer than that of TB alone to resolve. This could be explained by high Mtb bacterial load which may occur during advanced immunosuppression, the additive effect of HIV-1 induced inflammation and/or the effect of ART initiation. Indeed We previously showed that Mtb load significantly impacts on the inflammatory profile of TB patients⁴⁻⁸. Systemic dissemination of Mtb, a phenomenon more frequently observed in HIV-1 co-infected TB patients, associates with an inflammatory profile distinct from that of pulmonary TB, and shows delayed response to ATT as characterized by network analysis⁸. Thus, network analysis is able to detect important underlying trends in the systemic immune profile of TB cohorts, providing insight into disease pathogenesis. The objective of our study was to employ network analysis to investigate the extent to which the HIV-1 induced immune response contributes to the plasma-inflammatory profile in pulmonary TB and the effect of ATT and ART on it, as this has not yet been studied comprehensively. We hypothesised that HIV-1 co-infection, ATT duration and ART mediated viral suppression would each significantly alter the observed immune profile in our cohort. To this end, we measured a comprehensive panel of plasma inflammatory markers in a cohort of HIV-1 infected and uninfected TB patients from South Africa and conducted in depth analysis of the inflammatory profile at various timepoints during ATT.

Materials and Methods

Ethics Statement

The University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee (HREC) approved the study (568/2012) and written informed consent was obtained from all study participants in the primary discovery cohort. The validation cohort involved hospitalized HIV-TB co-infected patients (UCT HREC 057/2013) who also provided written informed consent when possible. Hospitalized patients who were eligible for study participation but could not immediately provide informed consent due to decreased level of consciousness were enrolled and followed up daily until they regained the capacity to participate in the informed consent process. UCT HREC approved the per protocol use of samples and information from those who died before providing informed consent, or who could not provide consent by the end of study follow up. HIV-1 infected healthy controls with no evidence of active TB were also enrolled (UCT HREC 057/2013) and provided written informed consent.

Primary discovery cohort

Participants aged 18 years or older with sputum Xpert MTB/RIF positive rifampin-susceptible pulmonary TB were recruited from at the Ubuntu Clinic, Site B Clinic, Khayelitsha, South Africa, as part of during a prospective cohort study to assess determinants of antituberculosis drug exposure and their role in development of acquired drug resistance (see Appendix 1 for eligibility criteria, clinical protocol and STROBE statement)^{3,9}. Our study We conducted a post-hoc analysis of plasma soluble-inflammatory markers measured at interval after ATT initiation, in the plasma of participants from this study, as well as that of the validation cohort and control participants described below. Patients were excluded from the primary discovery cohort if they were under the age of 18, unable to commence ATT for any reason, pregnant, had unknown HIV-1 status, were unable to consent to study participation, were unable to provide baseline sputum samples, have had more than 3 doses of ATT or

were being re-treated for TB within 6 months of their previous ATT regimen. Those who remained Mtb culture positive at week 8 were asked to donate plasma at week 20 (n=53). In addition, one participant with HIV-1 coinfection exhibited values below the detection range for more than > 20% of the analytes and thus was excluded from the Luminex analysis. The University of Cape Town (UCT) Faculty of Health Sciences Human Research Ethics Committee (HREC) approved the study (568/2012) and written informed consent was obtained from all study participants.

Disease extent on chest radiograph was assessed by experts and designated as either “extensive disease” where involvement of > one lung or involvement of ≥ one out of three zones per lung was evident or “mild disease” where only one zone of a lung was involved. All treatment failures, deaths during TB treatment, and true or possible TB recurrences were classified as “unfavorable outcomes”. Participants were investigated for disseminated TB to the extent that it would change their management, granted that they all had culturable Mtb from their sputum. This study was conducted in accordance with a clinical protocol (S1 document) that had received all relevant regulatory approvals and is reported according to the STROBE statement (S2 document).

Validation cohort and control group: clinical characteristics

The validation cohort was part of a larger cohort study¹⁰ that was recruited from 16th January 2014 until 19th October 2016 from patients hospitalized at the Khayelitsha Hospital, Cape Town (see supplementary table 1). HIV-1 infected patients with a CD4⁺ count below 350 cells/μL and a high clinical suspicion of new TB were enrolled. Only “microbiologically confirmed TB” cases (at least one culture or Xpert MTB/RIF test positive for Mtb from any clinical sample) and those with “probable TB” were included in the final analysis (see Appendix 1 for eligibility criteria). “Probable TB” was classified as those who, in absence of microbiological confirmation, had a positive Alere urine lipoarabinomannan test result and/or had a clinical and radiological picture in keeping with TB, were treated for TB and had no alternative diagnosis made during index admission. Exclusion criteria for the validation cohort were a positive pregnancy test, those who received ATT within the previous month and those who already commenced ATT and had received three or more doses. As part of this study a control group of HIV-1 infected outpatients with CD4⁺ counts below 350 cells/μL and no evidence of TB (asymptomatic, sputum Xpert MTB/RIF negative and no radiographic evidence of TB) were also recruited from the Site B Ubuntu Clinic, Khayelitsha. All participants (UCT HREC 057/2013) who also provided written informed consent when possible. Hospitalized patients those who were eligible, for study participation but could not immediately provide informed consent due to decreased level of consciousness were enrolled and followed up daily until they regained the capacity to participate in the informed consent process. UCT HREC approved the study (UCT HREC 057/2013) and the per-protocol use of samples and information from those who died before providing informed consent, or who could not provide consent by the end of study follow-up. HIV-1 infected healthy controls with no evidence of active TB were also enrolled (UCT HREC 057/2013) and provided written informed consent. Further details, including reporting according to the STROBE statement is available in the publication by Schutz et al.¹⁰. The three study groups, overall study flow and network analysis is summarized by Supplementary Fig. 1.

Role of Funding Source

The funder of the study played no role in study design, data collection, data analysis, data interpretation, or writing of the report. We have not been paid to write this article by a pharmaceutical company or other agency. All authors had full access to all the data in the study and agreed with the the decision to submit for publication.

Procedures

All participants recruited to the primary discovery cohort, the validation cohort and the outpatient control group underwent standardized study-specific clinical interview and examination after enrolment. Anonymised data was collected from the patient, their clinical/hospital folder and by review of relevant clinical laboratory results and captured on a access controlled, secure database. Baseline microbiological testing for Mtb (e.g. Xpert MTB/RIF, sputum smear for acid fast bacilli and Mtb culture), CD4 count and Viral Load testing was performed by the National Health Laboratory Services (NHLS). Data was entered on paper case report forms by investigators and captured on a access-controlled secure database. Venous blood samples were collected at enrolment from all participants, at week 8 of ATT from discovery cohort participants, and at week 20 of ATT from those in the discovery cohort who remained sputum culture positive at week 8. A venous blood sample was collected by a trained phlebotomist from all participants at the time of study enrolment. This was repeated in all discovery cohort participants at week 8 of ATT, with repeat sampling at 20 weeks of ATT in those who remained sputum culture positive at week 8 (n=53). Blood samples underwent laboratory processing, plasma was isolated and cryopreserved in -80°C freezers until batched extraction for immunoassays. Cryopreserved plasma samples were used in Luminex assays to quantify concentrations of several proteins and surrogates of systemic inflammation, immune activation and tissue remodeling. Data were extracted and Spearman correlation matrices for each clinical subgroup and timepoint were calculated (see Immunoassay Analysis below). There were no pre-specified

outcomes for our study as it was our intention to use network analysis to conduct an unbiased investigation into the effect of HIV-1 coinfection on the plasma inflammatory profile in pulmonary TB and how this was influenced by ART and ART status.

Immunoassays

We evaluated a panel of 39 cytokines, acute phase proteins and soluble receptors to examine inflammation and immune activation using different immunoassays in plasma samples. Marker selection was based on their potential role in TB pathogenesis as shown by previous work from our group^{6, 11} and others¹², and included: C-reactive protein (CRP), fibrinogen, ferritin, procalcitonin, serum amyloid protein A (SAA), serum amyloid protein P (SAP), alpha-2-macroglobulin, haptoglobin, tissue plasminogen activator (tPA), matrix metalloproteinase (MMP)-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, TIMP-3, TIMP-4, interleukin (IL)-1 α , IL-1 β , IL-1Ra, IL-6, IL-8, IL-10, IL-12p70, IL-17A, interferon (IFN)- α 2, IFN- γ , tumor necrosis factor (TNF)- α , CCL3, CCL4, CCL11, CXCL10, VEGF, sCD14, and 8-hydroxy-2'-deoxyguanosine (8-OH-dG). Luminex kits for the main cohort were used as follows: (i) C-reactive protein (CRP), fibrinogen, ferritin, procalcitonin, serum amyloid protein A (SAA), serum amyloid protein P (SAP), alpha-2-macroglobulin, haptoglobin and tissue plasminogen activator (tPA) were measured using the Bio-Plex Luminex kits from Bio-Rad (Hercules, CA); (ii) matrix metalloproteinase (MMP)-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10 and MMP-12 as well as tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, TIMP-3 and TIMP-4 were measured using Luminex kits from R&D Systems (Minneapolis, MN); (iii) interleukin (IL)-1 α , IL-1 β , IL-1Ra, IL-6, IL-8, IL-10, IL-12p70, IL-17A, interferon (IFN)- α 2, IFN- γ , tumor necrosis factor (TNF)- α , CCL3, CCL4, CCL11, CXCL10 and VEGF were measured using a Luminex kit from Millipore (Merk, Burlington, MA). Plasma levels of sCD14 were measured using ELISA (R&D Systems, Quantikine). Concentrations of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) were assessed by an ELISA from Cayman Chemical (Ann Harbor, MI). Please refer to Appendix 1 for the raw immunoassay data.

Immunoassay analysis

In table 1 and 2 median $\pm$ interquartile ranges were used as the measures of central tendency and dispersion respectively. The Mann-Whitney U test (for two groups) and was used to compare HIV-1 infected versus uninfected groups in table 1 as well as comparing CD4 T-cell count and HIV RNA copies/mL by ART status in supplementary figure 5, whilst the Kruskal-Wallis test was used for group and timepoint comparisons to compare the different subgroups of the validation cohort and outpatient controls. The Fisher's exact test (2x2 comparisons) and the Pearson's chi-square (other types of comparisons) were used to compare variables displayed as percentages in these tables.

A summary table listing all statistical tests, study timepoints and relevant cohorts used to construct the figures can be found in Appendix 1 the supplementary data (supplementary table 2). Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad, La Jolla, CA), STATA 11 (StataCorp, College Station, TX), JMP 14.0 (SAS, Cary, NC) and R 3.1.0 (R Foundation, Vienna, Austria) programs.

Hierarchical cluster analyses (Ward's method), with 100X bootstrap¹³ of log10 transformed and z-score normalized data were employed to depict the expression profile of all plasma biomarkers at the various study timepoints [baseline (week 0), week 8 and week 20 of ART] in the discovery cohort (Fig. 1A). This was followed by hierarchical clustering by both timepoint and HIV-1 status (Fig. 2A) as well as by ART status in the HIV-1 infected subgroup of the discovery cohort (Supplementary Fig. 5A). Dendrograms display the cluster hierarchy represented by Euclidean distances.

Venn diagrams were used to illustrate differentially expressed markers at the different each study timepoints in the discovery cohort¹⁴. The first Venn diagram (Fig. 1B) displays all markers that were differentially expressed between baseline and follow-up timepoints, whilst the following Venn diagram displays differentially expressed markers by both timepoint and HIV-1 status (Fig. 2B).

Fold change (Log10) of plasma markers was calculated where relevant for HIV-1 infected compared to uninfected participants (Fig. 2A), as well as those who were ART established compared to ART naïve (supplementary figure 5A) at the various study timepoints in the discovery cohort. Differences with p values below 0.05 after Holm-Bonferroni's adjustment for multiple comparisons were considered statistically significant. All comparisons were pre-specified and two-tailed.

The Spearman rank test was used to assess all correlations between all plasma markers and other variables in the discovery cohort (see below: Network analysis), as well as to investigate their correlation with CD4 T-cell count and HIV RNA copies/mL in the HIV-1 infected subgroup of the discovery cohort (Fig. 2C).

Network analysis

Profiles of correlations between biomarkers in different subgroups and timepoints were examined using network analysis of bootstrapped Spearman correlation matrices. We displayed correlation matrices as heatmaps and visualized the networks using circular layout network plots using Gephi v. 0.82 with circular layout plug-in. Only correlations with a p-value smaller than 0.05 in at least 80 iterations of 100 bootstraps were included in network visualization. Network density was established calculating the actual connections (known number of connections between nodes) by potential connections (number of potential connections between two nodes; appendix 1; supplementary figure 1). Node analysis was conducted to calculate the number of connections per molecule (the arcs are defined as a significant correlation, $p < 0.05$) by timepoint and build a curve and ANOVA linear trends test to assess variable dependency. The area under the curve (AUC) was used to measure variation between connection numbers over time and identified molecules with most connections.

In the discovery cohort, network analysis was applied to investigate biomarker expression in the overall cohort at different timepoints and to assess the effects of TB and HIV-1 co-infection, ART, and HIV-1 viraemia on biomarker expression.

Network analysis was also used to measure IL-17A network connectivity in the validation and control cohorts. The numbers of connections with IL-17A were compared between: those who died versus survived in the validation cohort; those with a CD4 count below 100 cells/ μ L versus a CD4 count of ≥ 100 cells/ μ L in the control cohort; and those with an HIV-1 viral load of ≤ 39 RNA copies/mL versus those with a viral load over 39 copies/mL. A receiver operating characteristic (ROC) curve was constructed to evaluate whether the number of connections with IL-17A could predict death in the validation cohort, or low CD4 count or high viral load in the control group. For further explanation of the network analysis procedure, please refer to appendix 1. Profiles of correlations between biomarkers in different subgroups and timepoints were examined using network analysis of bootstrapped Spearman correlation matrices (Supplementary Fig. 2, 4 and 6). Only strong correlations, defined as having a significant p-value (< 0.05) in at least 80+ iterations of 100 bootstraps, were included in network visualization. The node analysis using replication of each network at different timepoints was performed by calculating the number of connections per molecule (the arcs are defined as a significant correlation, $p < 0.05$) by time point and network density (ND) was established calculating the actual connection [known connections number between nodes (AC)] by potential connection [A connection number that could potentially exist between two nodes (PC)]:

$$PC = n * (n - 1) / 2$$

$$\text{Network Density} = AC / PC$$

AC = Actual Connections

PC = Potential Connections

n = Number of nodes

The Networks figures were designed in Gephi™ 0.82 with circular layout plug-in (further overview of this is provided in supplementary figure 1).

Network analysis of plasma inflammatory markers measured in the discovery cohort at the study timepoints are thus visualized as circular layout network plots (Fig. 1C, Supplementary Fig. 3A, Supplementary Fig. 7A, Fig. 3A and Supplementary Fig. 10A) with the direction of each correlation (positive or negative) depicted in the correlation matrices, displayed as heatmaps (Supplementary Figures 2, 4 and 6). Correlation matrices were built for each timepoint and were submitted to 100x bootstrap. The heatmaps show correlations that persisted with a significant p-value (false discovery rate of 5%) in at least 80 times out of 100 bootstraps.

Next, overall network density per study timepoint was calculated as described above (Fig. 1D, Supplementary Fig. 3B, Supplementary Fig. 7B, Fig. 3B and Supplementary Fig. 10B), with the top markers in terms of statistically significant correlations at each timepoint and for each comparison group being identified (Fig. 1E, Supplementary Fig. 3C, Supplementary Fig. 7C, Fig. 3C, Supplementary Fig. 10C). Data were compared between groups and timepoints (for summary see supplementary table 2) using the Kruskal-Wallis test with Dunn's multiple comparisons *ad hoc* test. The comparison between network densities was established by permutation test with 10000x permutation developed in native R. Each marker was considered individually, with comparison being made between the amount of statistically significant correlations with other plasma markers for each comparator group, at each timepoint (Friedman's matched pairs test with Dunn's multiple comparisons *ad hoc* test or non-parametric linear trend analysis). In addition, at each timepoint and in each of the study groups the number of connections were used to build a curve and ANOVA linear trends test to assess variable dependency. The area under the curve (AUC) was used to measure variation between connection numbers over time and identified molecules with most connections overall for the three study visits (Fig. 1F, Supplementary Fig. 3D, Supplementary Fig. 7D, Fig. 3D and Supplementary Fig. 10D).

Finally, we turned to the validation cohort to measure IL-17A network connectivity in a hospitalised cohort of HIV-TB patients (Fig. 4) and compared the number of connections with IL-17A between those in the cohort who died and those who survived (Fig. 4A). A receiver operating characteristic (ROC) curve was constructed to evaluate whether the number of connections with IL-17A could predict death in the validation cohort (Fig. 4B). The optimal cut-off value was determined and the percentage of expected true positive, false positive, true negative and false negative results using this cut-off are visually displayed in Fig. 4C. The same approach was used in the HIV-1 infected, non-TB outpatient control group where the number of connections with IL-17A during network analysis were compared between those with a CD4 count below 100 cells/μl and those with a CD4 count greater than or equal to 100 cells/μl (supplementary figure 9A). This was repeated in the same control group, this time comparing those with an HIV-1 viral load equal to or below 39 RNA copies/mL with those with a viral load (VL) over 39 copies/mL (supplementary figure 9B).

For further detailed explanation of the network analysis procedure, please refer to appendix 1.

Logistic regression and k-fold cross validation models

We performed binary logistic regression modelling to identify patient characteristics aside from HIV infection status that may be associated with increased network connectivity/density in the discovery cohort (supplementary table 3). In the first instance the model was used to test association between the parameters and increases of 1 unit in network density (connectivity) of the entire study population. The resampling was performed to evaluate the model on the data sample, using a parameter called “k” that refers to the number of groups the data sample was split into. One proportion of the data was used as training dataset and the other as validation set thus using a limited sample to infer how the model is expected to perform when used to make predictions on data not used during the training of the model. The k (10) Fold Cross Validation was performed using classification and Regression Training package (CARET) available in R. Of all the variables analysed, including male sex, age, weight, smoking, HIV-1 infection, detection of acid-fast bacilli in sputum, presence of cavities and sputum culture conversion at week eight, only HIV-1 infection and the presence of cavities on chest radiograph significantly associated with increased inflammatory network connectivity (supplementary table 3).

A similar statistical model was employed to test whether IL-17A connectivity was associated with death after adjustment for potential confounding variables in the validation (supplementary table 4). Thus, binary logistic regression with k-fold cross validation modeling, as described in the aforementioned, was employed to evaluate for the possible effect of microbiologic confirmation of TB, male sex, age, weight and IL-17A network connectivity on mortality in the validation cohort (supplementary table 4). Of all these parameters, only IL-17A network connectivity was significantly associated with mortality.

We used binary logistic regression modelling to identify patient characteristics that may be associated with increased network connectivity/density in the discovery cohort and to test whether IL-17A connectivity was associated with death after adjustment for potential confounding variables in the validation cohort (see supplementary tables 3 and 4; appendix 1).

Results

The discovery cohort consisted of 129 participants (see table 1) of which 76 were HIV-1 coinfecting, with a median CD4 count of 192 (IQR: 67.5-365) and median viral load of 108692 copies/mL (IQR: 126-385633) at study enrolment. Of the HIV-1 infected participants 29 (38.2%) were on ART and 20 (26.3% of all HIV-1 infected) of these participants being were virally suppressed (HIV VL < 40 copies/mL) at enrolment. ART was commenced between 2 and 8 weeks after ATT initiation in HIV-1 infected patients as per national protocol in South Africa. By week 8 71% of the HIV-1 infected cohort had commenced ART. The timepoint of ART initiation was at the discretion of the treating clinician at the Site B Clinic and not standardized, however most participants commenced ART between 2 and 8 weeks after ATT initiation as per national guidelines in South Africa, with 71% (n=54) of the HIV-1 infected cohort being on ART by week 8. The overall age distribution of the HIV-1 infected and uninfected subgroups were similar, [median 35.1 (IQR: 30-43.7)] however there were more males in the HIV uninfected subgroup [77.4 versus 43.4% (p<0.01)] and the median body mass index (BMI) of the HIV-1 uninfected subgroup was slightly lower [20 vs 22 kg/m² (p=0.037)]. As expected HIV-1 coinfection was associated with significantly less radiological evidence of “extensive disease” [55.3 versus 92.5% (p<0.001)] and a trend towards less cavitation [44.7 versus 62.3% (p=0.075)], whilst their sputum smear was more commonly negative/scanty positive (47.4 versus 16.9%) than HIV-1 uninfected participants who tended towards higher smear grades (2+ or 3+) at study enrolment (64.2% versus 32.9%). Of all the clinical variables recorded, including

male sex, age, weight, smoking, HIV-1 infection, detection of acid-fast bacilli in sputum, presence of cavities and sputum culture conversion at week 8, only HIV-1 infection and the presence of cavities on chest radiograph significantly associated with increased inflammatory network connectivity by binary logistic regression modelling (supplementary table 3).

We examined the expression of inflammatory markers in TB patients before starting ATT (n=129), at eight weeks (n=129) and at 20 weeks (n=53) of ATT in the discovery cohort. Hierarchical cluster analysis revealed a distinct plasma inflammatory marker expression profile at each of the timepoints before and during ATT (supplementary Fig. 4A2A). Although the pre-treatment timepoint was characterized by predominantly higher expression of most inflammatory markers accompanied by a decrease over the course of ATT, there were a number of markers that did not follow this pattern. To explore this further we compared the expression of these markers at week eight and week 20 to the pre-treatment timepoint and identified markers that displayed statistically different levels (Fig. 4B1A). The majority of the markers showed an early and sustained decrease on ATT.

We used correlation matrices (supplementary Fig. 23) and circular layout network plots to visualize differences in correlation profiles between the inflammatory markers at the various timepoints and calculated overall network density (Fig. 4C-1B and supplementary Fig. 2DB). Network density was highest pre-treatment and was not significantly different at week eight; however a dramatic decline was observed at week 20 (Fig. 4C1B).

We conducted node analysis of the top nine markers with the greatest statistically significant correlations per timepoint to define inflammatory markers driving overall network density (Fig. 4E1C). Considering the number of statistically significant connections for each marker at the three timepoints we calculated the AUC for each and ranked the markers in order of their relative contribution to network density. The AUC value thus reflects the overall capacity of the marker to correlate with others in the network over the three study timepoints. The marker with most network connections was IL-17A, which in turn had a significantly higher AUC than all the other ranked markers (supplementary Fig. 4F2C).

Next we investigated whether HIV-1 co-infection perturbed the plasma inflammatory profile of TB patients. Hierarchical clustering revealed significant differences in inflammatory marker levels between HIV-1-infected (n=76) and -uninfected (n=53) TB patients at different timepoints, which were quantified by calculating fold-change (supplementary Fig. 4Fig. 2A). Differences were greatest at the pre-treatment timepoint where six inflammatory markers were significantly lower in HIV-TB co-infected versus HIV-1-uninfected TB patients and seven were significantly higher. When comparing differentially expressed markers at the pre-treatment timepoint versus week eight and week 20 on treatment respectively, the majority were decreased in both HIV-1-infected and -uninfected TB patients (Fig. 2B2A).

Upon assessing significant correlations of plasma inflammatory markers in the HIV-1 infected subgroup with either CD4 count or HIV-1 viral load we found most markers to show significant negative correlation with CD4 count and significant positive correlation with HIV-1 viral load (Fig. 2C2B).

Considering the possibility that HIV-1 co-infection may have a substantial effect on individual plasma inflammatory marker expression in TB patients, we investigated how this influenced correlations between these markers. Correlation matrices of HIV-1 co-infected versus -uninfected participants provided evidence of differential correlation between the inflammatory markers in each of these subgroups and timepoints (supplementary Fig. 3-5 and supplementary Fig. 46). Network analysis revealed significantly greater density in the HIV-1 co-infected compared to the HIV-1-uninfected group pre-treatment and at week eight, with no significant difference being observed at week 20 (supplementary Fig. 3A-5A and B). A linear decrease in network density was observed in HIV-1 co-infected participants, whereas HIV-1-uninfected participants showed slightly increased network density compared to pretreatment at week eight, with significant decrease only occurring at week 20. The top 3 markers contributing most to overall network density by AUC in the HIV-1-uninfected subgroup were CXCL10, CRP and IFN- α 2, whereas the top 3 markers in the HIV-1 co-infected subgroup were IL-17A, IL-1 β and TNF- α (supplementary Fig. 3C-5C and D). IL-17A was the most, and TNF- α the third most influential markers in network connectivity analysis of the cohort as a whole.

To further investigate the effect of HIV-1 on the plasma inflammatory profile we assessed the impact of ART on network connectivity in HIV-TB co-infection. Most participants in the ART established group were virally suppressed with 20/29 (68.9%) having viral loads below the limit of detection (<40 copies/ml) and a trend towards higher median CD4 T cell count than that of the ART naïve subgroup [272 (IQR: 107-371) cells/ μ l vs 128 (IQR: 53-355) cells/ μ l] (supplementary Fig. 5B7B). Although ART status influenced the expression of some of the

inflammatory markers in HIV-TB co-infected participants at various timepoints there was no statistically significant difference between the ART established ($n=29$) and naïve ($n=47$) subgroups in terms of frequency of extensive disease on chest radiograph, culture positivity at week eight⁸ and unfavorable outcomes (supplementary Fig. 5A-7A and 5B).

Despite only modest differences in individual plasma marker expression being observed between ART naïve and established co-infected participants (supplementary Fig. 5A-7A), network analysis of correlation matrices (supplementary Fig. 6B) for each timepoint pre- and post-ATT showed marked differences (supplementary Fig. 7A-9A). Pre-treatment network density was significantly higher in ART naïve participants compared to those established on ART (supplementary Fig. 7B-9B). Strikingly, similar network density to that of the pre-treatment timepoint was observed in the ART naïve group despite eight weeks of ATT, contrasting with the ART established group at week eight⁸ who displayed significantly decreased network density. At week 20 both ART naïve and established groups displayed similarly low network density. Nodal analysis revealed that IL-17A again demonstrated the greatest nodal connectivity in ART naïve co-infected participants, with IL-1 β and TNF- α in second and third place respectively (supplementary Fig. 7C-9C). In the ART-established group IFN- α 2, IL-17A and IL-1 β were the markers with greatest nodal connectivity (supplementary Fig. 7D-9D).

Not all ART established participants were virally suppressed and we hypothesized that HIV-1 viraemia contributed to their observed inflammatory profile. Upon stratifying HIV-1-infected participants by those who had suppressed ($n=20$) versus unsuppressed ($n=56$) viral loads and employing inflammatory network analysis of correlation matrices (supplementary Fig. 8-10), we found that this was indeed the case (supplementary Fig. 11A-11C and Fig. 3B-3C). Those who had suppressed HIV-1 viral loads had comparable low network density at all timepoints, including pre-treatment. Furthermore, those who were viraemic exhibited high network density pre-treatment, no statistically significant change in density at week eight⁸ with significant decrease in network density only occurring at week 20. Nodal analysis again identified IL-17A as the most connected node in those with unsuppressed HIV-1 viral loads (Fig. 3C-3B and 3C).

Finally, we applied network analysis to two validation studies: one to investigate mortality in hospitalized HIV-TB co-infected patients and the other examining HIV progression in the absence of active TB (see supplementary table 1). The hospitalized HIV-1 co-infected cohort¹⁰ with available plasma samples consisted of 516 patients admitted with probable or definite TB, of which 113 died within 12 weeks of enrolment. There were 29 participants in the outpatient control group who were HIV-1 infected without evidence of TB. Those of the hospitalized group who died tended to be slightly older [median age: 39.34 (IQR: 32.10-46.74)] than those who survived [median age: 35.5 (IQR: 30.5-42.1)] and those in the outpatient control group [median age: 34.2 (IQR: 29.9-41.5)]. There were 272 females (52.7%) and 244 males (47.3%) in the hospitalized cohort, with similar sex distribution by subgroup. The outpatient control group were predominantly female (21/29, 72.4%). Those of the hospitalized cohort who died tended to have lower median CD4 counts [39 (IQR: 17-88)] than those who survived [64 (IQR: 23-126.5)] and the outpatient controls [159 (IQR: 74-204)]. The majority of the hospitalized cohort was not on ART with only 186 (36%) being on ART at enrolment. ART data is not available for the outpatient control group. Of the hospitalized subgroup that had sputum Xpert-MTB/RIF results available 79.7% (59/74) of those that died and 66.1% (218/330) of those that survived tested positive. The majority of participants in the hospitalized cohort had radiographic evidence of TB (482/500, 96.4%).

In the hospitalized co-infected cohort¹⁰ we found the number of connections with IL-17A to be significantly higher in those who died ($n=113$) compared to those who survived ($n=394$) ($p=0.02$, Fig. 4). Binary logistic regression modelling confirmed that this association was not confounded by the effect of microbiologic confirmation of TB, weight, male sex or age (supplementary table 4). The number of connections with IL-17A through network analysis exhibited 88.0% sensitivity and 89.0% specificity to differentiate those who died versus survived hospital admission for HIV-1-associated TB. In the HIV-1 infected outpatient control group without active TB ($n=29$) the number of nodal connections with IL-17A was significantly higher in those with lower CD4⁺ counts (supplementary Fig. 9A-12A) and unsuppressed viral loads (supplementary Fig. 9B-12B). Conversely, network analysis of the primary discovery cohort showed that TB infection in the absence of HIV-1 co-infection contributed little to IL-17A network connectivity, even in those with extensive TB disease on chest radiograph (supplementary Fig. 10-13).

Discussion

We performed detailed analysis to better understand the effect of HIV-1 co-infection on the systemic-inflammatory profile of a well characterized cohort of TB patients undergoing ATT. By analyzing the discovery cohort as a whole we did not find or observe any decline in the plasma network density between pre-treatment and week eight⁸.

8 of ATT, significant decline in density was only observed at week 20 in the discovery cohort. This could not be explained by delayed culture conversion, a factor known to significantly influence the plasma inflammatory profile⁴⁻⁸, as the majority of participants were no longer sputum culture positive at week eight⁸. Importantly, stratification by HIV status revealed that network density in the cohort as a whole was driven by that of the HIV-1 coinfecting subgroup, owing to much greater network density observed at week 8 than that of HIV-1 uninfected TB patients. When we further stratified the HIV-1 coinfecting subgroup by ART status we found that the ART naïve inflammatory profile seemed to underlie the high network density we had observed in the greater HIV-1 coinfecting group, thus potentially offering explanation for the delayed decline in network density in the cohort as a whole. The majority of the HIV-1 infected group were not on ART at enrolment (61·8%), with 29% yet to commence ART by week 8. Thus, in absence of availability of viral load data at week 8, and considering that many HIV-1 infected participants were newly commenced on ART or still ART naïve at this timepoint, it can be assumed that a sizable proportion of the HIV-1 coinfecting group would still have been viraemic at week 8. Dissecting this effect further we showed that HIV-1 viraemia was driving network connectivity in HIV-TB co-infected patients and that the overall immune profile of those who were virally suppressed exhibited minimal network density. Through network analysis of key plasma-inflammatory mediators we have thus found that HIV-1 viraemia during HIV-TB coinfection mediates ongoing systemic inflammatory activity, with ART mediated viral suppression abrogating this effect.

Little is known about the individual contribution of HIV-1 and TB to systemic immune activation and inflammation observed during co-infection. In vitro models of macrophage co-infection suggest that Mtb-derived gene expression predominates over that of HIV-1, with particular upregulation of pro-inflammatory pathways and type I interferon (IFN) gene signalling¹⁵. Conversely, productive infection of monocyte-derived macrophages with HIV-1 only did not activate inflammatory pathways and was not associated with interferon gene expression¹⁶, which was later shown to be part of an HIV-1 mediated immune evasion strategy¹⁷. However, our ex vivo findings indicate that HIV-1 viraemia may play a greater role in promoting inflammation in HIV-TB co-infection than previously appreciated.

Another important hypothesis generating finding was that IL-17A was identified as the most important node during network analysis in the cohort as a whole, as well as in the ART naïve and HIV-1 virally unsuppressed subgroups. Furthermore, IL-17A was the second most connected node in the ART-established and the HIV virally suppressed groups, but did not feature in the ranking of the top influential nodes in HIV uninfected TB patients. This implicates IL-17A and its related immune networks in the inflammatory response to HIV-TB, especially in the setting of unsuppressed HIV-1 viral load. To further confirm and elaborate on our findings we turned to the hospitalized HIV-TB coinfecting cohort and HIV-1 infected outpatient control group. By applying network analysis to the hospitalized HIV-TB coinfecting cohort we found that the overall number of connections with IL-17A differentiated those who died and survived hospital admission for HIV-1 associated TB with high sensitivity and specificity. We show that in an HIV-1 infected outpatient control group without TB, advanced HIV-1 as denoted by low CD4 counts (CD4 count < 100 cells/mL) and high viral loads (VL > 39 copies/mL) was associated with higher IL-17A network connectivity, similar to those who died of HIV-TB. By contrast, evidence of severe TB disease on chest radiograph was not associated with markedly increased IL-17A network connectivity. IL-17A network connectivity thus serves as a read-out of higher systemic inflammation and immune activation induced by advanced HIV-1 infection and uncontrolled HIV-1 viral replication and denotes higher mortality risk in HIV-TB co-infection.

Little is known about the individual contribution of HIV-1 and TB to systemic immune activation and inflammation observed during co-infection. In vitro models of macrophage co-infection suggest that Mtb-derived gene expression predominates over that of HIV-1, with particular upregulation of pro-inflammatory cytokines and chemokines, their receptors and type I interferon (IFN) gene signalling¹⁵. Conversely, productive infection of monocyte-derived macrophages with HIV-1 only did not activate inflammatory pathways and was not associated with interferon gene expression¹⁶, which was later shown to be part of an HIV-1 mediated immune evasion strategy¹⁷. However, our ex vivo findings indicate that HIV-1 viraemia may play a greater role in promoting inflammation in HIV-TB co-infection than previously appreciated.

IL-17A was identified as the most connected node during network analysis in the cohort, as well as in the ART naïve and HIV-1 viraemic subgroups. IL-17A is produced by various cell types with widespread expression of its receptor being observed and its a cytokine with pluripotent effects ranging from but is characterized by its important role in mucosal barrier protection to immunopathology in autoimmune disorders and repair whilst conversely being known to mediate immunopathology in autoimmune disorders¹⁸. IL-17A is produced by a number of innate and adaptive cells including T helper 17 (Th17) cells, with its receptor being expressed

ubiquitously on different cell types¹⁹. There is some evidence that IL-17 may play a beneficial role in TB by plays a beneficial role in TB, especially early after infection in contributing to granuloma maturity, however IL-17 may it can also induce contribute to tissue damage through induction of MMPs²⁰⁻²². Most studies report higher IL-17 production in latent TB infection than in active TB²³, and thus there is minimal existing evidence of the induction IL-17 related cytokine networks by *Mtb* *Mycobacterium tuberculosis* per se. Indeed, our findings indicate that increased IL-17A network connectivity is mediated by HIV-1 viraemia and not TB. HIV-1-Tat protein has been shown to induce transcriptional dysregulation with particular activation of IL-17 signalling pathways, induction of T cell activation and increased pro-inflammatory cytokine secretion, including IL-17, being confirmed at the protein level²⁴. Furthermore, Th17 cells are preferentially depleted by HIV-1 owing to their abundant expression of co-receptors enabling HIV-1 entry and by being permissive to productive HIV-1 infection²⁵. Thus a profound here is early depletion of IL-17 producing Th17 cells, are observed at mucosal barriers early after HIV-1 infection, with leading to the gut mucosa becoming compromised and prone to microbial translocation contributing to chronic immune activation and disease progression²⁶. The combination of ongoing mucosal inflammation, HIV-1 replication and *Mtb* co-infection may result in heightened immune activation leading to constant triggering of the remaining IL-17A producing cell populations during HIV-TB co-infection. This is exemplified by findings of upregulated Th17 polarizing cytokines and transcription factors in pleural fluid of HIV-TB co-infected patients with TB pleurisy, despite numeric depletion of Th17 cells^{27,28}. Indeed it has been shown that IL-17A interacts synergistically synergises with other cytokines, especially TNF- α and IL-1 β to mediate induction of IL-6 and IL-8 and promotes the synthesis of pro-inflammatory cytokines such as TNF- α and IL-1 β ^{28,29}. Moreover, IL-17 itself has been shown to promote synthesis of TNF- α and IL-1 β in macrophages, with multiple other cytokines being similarly induced^{30,31}. IL-17A thus displays pleiotropic capacity to influence cytokine signalling and can activate diverse signalling pathways including nuclear factor κ -light chain enhancer of activated B cells (NF- κ B) shared by many other pro-inflammatory cytokines¹⁸. These immunological relationships coordinated by IL-17A are clearly reflected through our network analysis findings, with IL-17A, IL-1 β and TNF- α featuring as the top 3 most influential nodes in our HIV-1-co-infected and ART naïve groups. This is reproduced in the top four most influential nodes of HIV-1-infected viremic participants, with IL-8 showing the second most numerous network connectivity after IL-17A, followed by IL-1 β and TNF- α .

Our study is limited by the fact that we do not know the approximate duration of HIV-1 infection of these participants, a factor that could affect which may have significant effect on their the inflammatory profiles. Furthermore, the baseline median CD4 count of the discovery cohort was 192 cells/ μ L (67.5-365 cells/ μ L), and thus it is difficult to anticipate to what extent our findings may be applicable to those with higher CD4 counts and the timing of ART initiation varied in the ART naïve, HIV-1 infected participants precluding analysis to assess for the effect of ART duration. Only those who were *Mtb* culture positive donated plasma at week 20, representing a bias inherent to the study design. (n=53) and this thus represents an inherent bias that has to be borne in mind when reviewing the results of the study. However, owing to their *Mtb* culture positivity at the end of the intensive phase one would expect these participants to represent those with the highest inflammatory signature of the cohort as a whole and it is thus significant to see the dramatic decrease in network connectivity from week 8 to 20 and further strengthen our findings that HIV-1 viraemia drives network connectivity during HIV-TB co-infection. There was variation in the timing of ART initiation in the ART naïve HIV-1 infected participants of the discovery cohort as this was determined by the treating clinicians. It was thus impossible to conduct subgroup analysis to assess for the effect of ART duration. We did not use laboratory based testing to actively exclude all possible other concomitant opportunistic infections, but as this would have been prohibitively expensive. We are confident however that the majority of these would have been detected by clinical review of participants. In this study we have measured We measured only soluble inflammatory markers, however further investigation of our findings by employing different complementary laboratory technologies such as flow cytometry based assays or transcriptome analysis could potentially provide further insight.

A comprehensive assessment of the inflammatory responses in the immune pathogenesis of HIV-TB coinfection remains challenging owing to numerous potential biomarkers and their complex interactions. Here we add to mounting evidence that network analysis represents a useful, rigorous statistical approach which allows not only allowing biomarker discovery, but also a means to identify and identification of immune pathways associated with disease severity and treatment outcome. Our findings not only highlight the capabilities of network analysis in large, complex datasets but also on a practical level reiterate the importance of timeous ART initiation, particularly in those with advanced HIV-1 and HIV-TB coinfection. While IL-17A and its capacity to orchestrate the inflammatory response through synergy with other cytokine related inflammatory networks have been extensively studied in autoimmune disease, here we show for the first time its integral role in HIV-TB co-infection, concurring with suggestions that. It has been suggested that autoimmunity may autoimmunity may represent be an overlooked component of the pathological immune response to TB³⁴ TB³⁰. Further studies are required to confirm whether our finding of upregulated IL-17A related inflammatory networks in HIV-TB are indeed associated with an auto-

641 immune response to TB, to elucidate the exact mechanism whereby IL-17A network connectivity is enhanced
642 during HIV-TB co-infection and how HIV-1 viraemia drives this effect. These mechanistic studies might ~~then~~
643 reveal whether IL-17A related inflammatory pathways are cause or consequence of detrimental outcomes in HIV-
644 1 and HIV-TB infection and thus potentially inform novel therapeutic targets ~~for therapies and host-directed~~
645 ~~therapies~~.

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649 **Conflict of Interest Statement**

650 The funders had no role in study design, data collection and interpretation, or the decision to submit the work for
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655
656 **Data Sharing Statement**

657 [Detailed description of network analysis methodology as well as the full clinical protocol is available in appendix
658 1. All raw Luminex immunoassay data is available in appendix 2.](#)

659
660 **Authors and Contributors**

661 EDB, NR, CS, KAW, BBA performed experiments; EDB, NR, KAW, CS, GM, CR, RJW, BBA designed
662 experiments; EDB, NR, KFF, CR, BBA analyzed the data; NR and CS conducted the clinical recruitment,
663 administered the projects and curated the respective datasets; KAW, GM, RJW provided materials and
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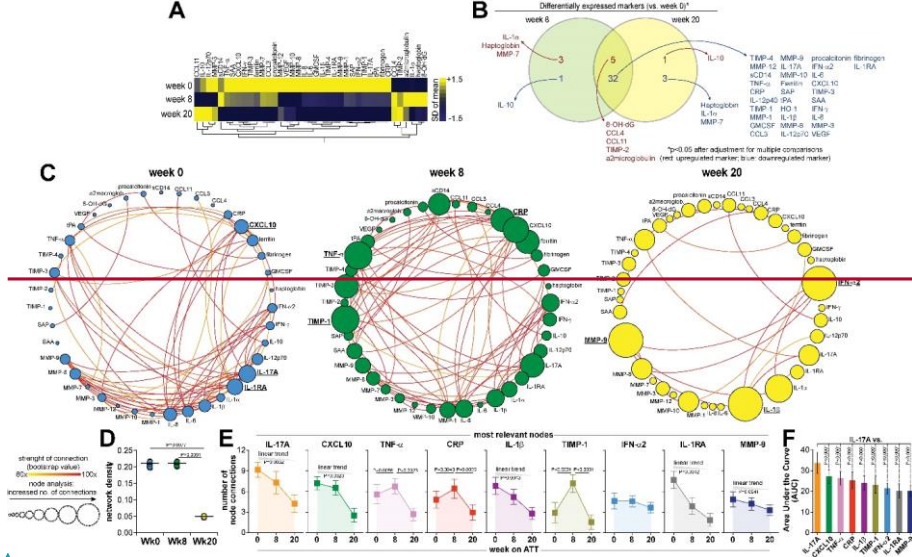
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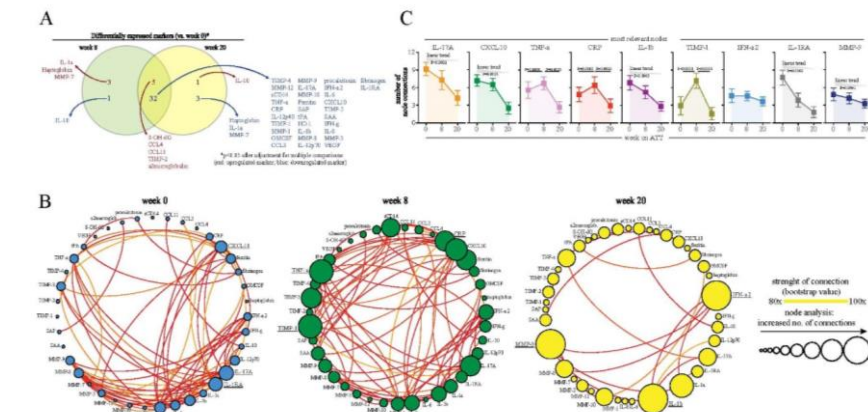
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Number on ART	-	29 (38.2)	-	-
Number virally suppressed (viral load < 40 copies/ml)		20 (26.3)	-	-

Data above reflect the number (%) except for age, BMI, CD4 and Viral load where the median value (interquartile range) is displayed. BMI: Body Mass Index. AFB: acid-fast bacilli



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Figure 1. Inflammatory signatures of plasma markers in pulmonary TB patients undergoing antitubercular therapy. Concentrations of several markers of inflammation, immune activation, tissue remodeling and oxidative damage were assessed in plasma samples from a cohort of 129 pulmonary TB patients before and at indicated timepoints after anti-TB treatment initiation. Luminex technology was used to quantify the markers. Data were log-transformed.

(A) Hierarchical cluster analysis using z-scored values of each parameter (Ward's method) was employed to depict the overall biomarker expression profile in the study population at the different timepoints.

(B)(A) Venn Diagram describes the markers which values were statistically different between week eight or week 20 and week 0 of antitubercular therapy.

(B) Network analysis of the biomarker correlation matrices was performed with bootstrap (100x). Relationships which remained statistically significant in at least 80 out of 100 bootstraps were plotted as

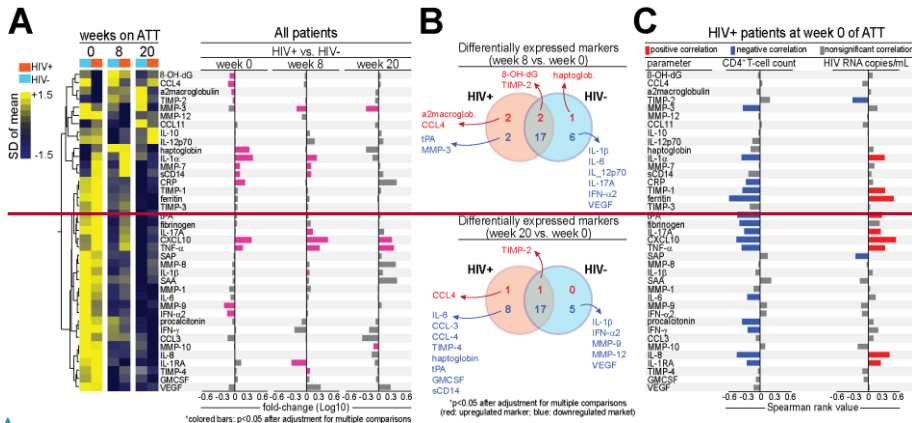
connecting lines. Each node represents a different plasma parameter. The size of each circle is proportional to the number of significant correlations involving such node in each network (sizes of the circles are balanced

for each network). The nature of each correlation (whether is positive of negative correlation) is described in Supplementary Figure 23.

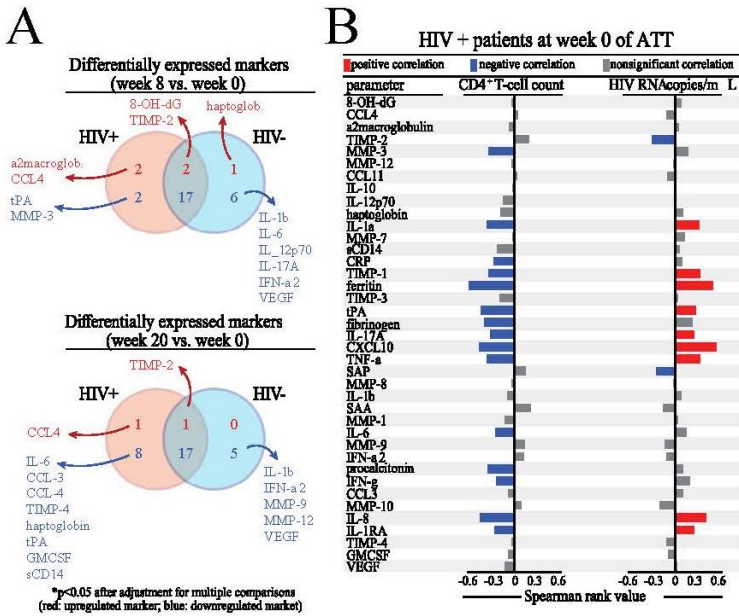
(D) Network densities of each bootstrap were calculated for each study group and timepoint as described in Materials and Methods. Data were compared using the Kruskal-Wallis test with Dunn's multiple comparisons *ad hoc* test.

(E)(C) At each timepoint, the top three markers with the highest number of statistically significant correlations were selected. The number of connections for each marker were compared between the study timepoints using the Freedman's matched pairs test with Dunn's multiple comparisons *ad hoc* test or non-parametric linear trend analysis. P-values were adjusted for multiple comparisons using the Holm-Bonferroni method.

(F) Area under the curves (AUC) values were calculated for each marker shown in (E).



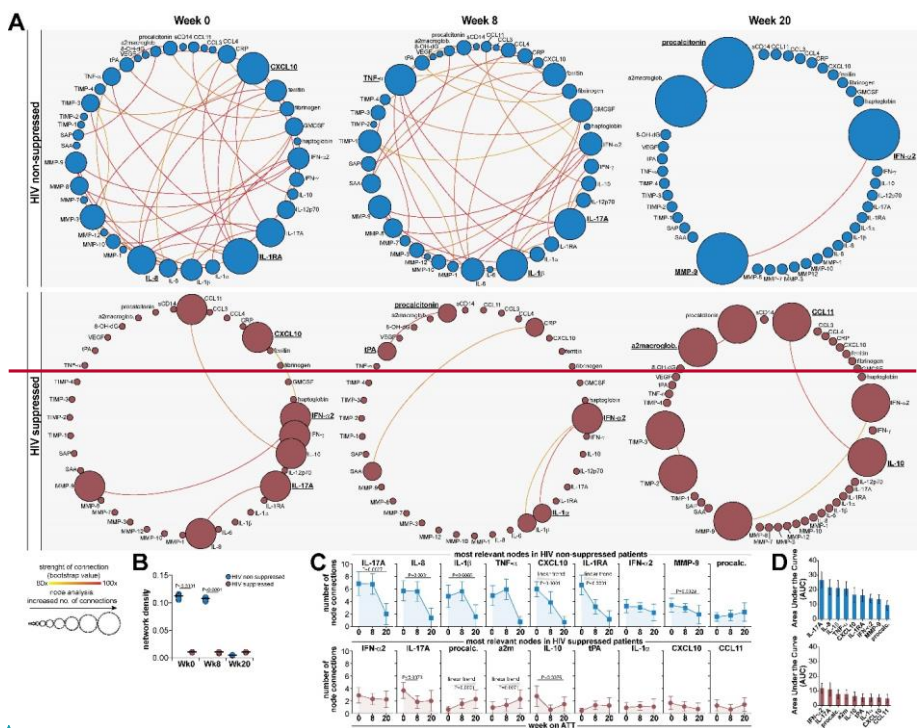
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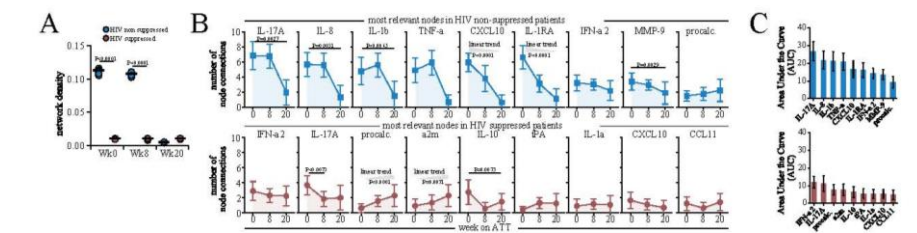
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Figure 2. Differential expression of plasma markers in pulmonary TB patients stratified according to HIV-1 infection status.

(A) Hierarchical cluster analysis using z-scored values of each parameter (Ward's method) was employed to depict the overall biomarker expression profile in the study population at the different timepoints of antitubercular therapy (ATT) and stratified according to HIV-1 infection status. Fold variation values (HIV-1 positive vs HIV-1 negative) were calculated and statistically significant differences of several biomarkers are highlighted in pink. (B) Venn Diagrams describe the markers whose values were statistically different between week eight or week 20 and week 0 of antitubercular therapy in subgroups of patients stratified according to HIV-1 infection status. (C) Plasma levels of the indicated markers measured at pre-ATT were tested for correlations with CD4+ T cell counts as well as HIV-1 RNA viral loads using the Spearman correlation rank test.



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Figure 3. Inflammatory signatures of plasma markers in pulmonary HIV-1-TB co-infected patients stratified by HIV-1 viral suppression during ATT.

(A) Network analysis of the biomarker correlation matrices was performed with bootstrapping (100x). Relationships which remained statistically significant in at least 80 out of 100 bootstraps were plotted as connecting lines. Each node represents a different plasma parameter. The size of each circle is proportional to the number of significant correlations involving such node in each network (sizes of the circles are balanced for each network). The nature of each correlation (whether positive or negative) is described in Supplementary Figure 8. (BA) Network densities of each bootstrap were calculated for each study group and timepoint as described in Materials and Methods. Data were compared using the Kruskal-Wallis test with Dunn's multiple comparisons *ad hoc* test. (CB) At each timepoint, the top three markers with the highest number of statistically significant correlations were selected. The number of connections for each marker were compared between the study timepoints using the Freedman's matched pairs test with Dunn's multiple comparisons *ad hoc* test or non-parametric linear trend analysis. P-values were adjusted for multiple comparisons using the Holm-Bonferroni method. (PC) Area under the curves (AUC) values were calculated for each marker shown in (C).

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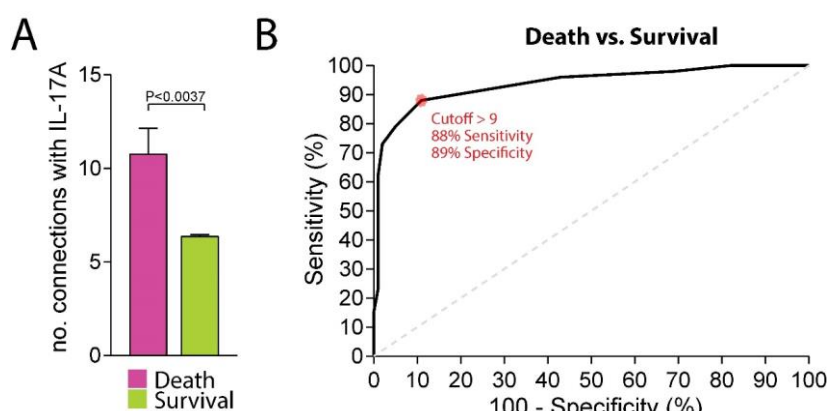


Figure 4. Inflammatory signatures of plasma markers in hospitalized pulmonary HIV-1-TB patients undergoing ART at week 0 of antitubercular therapy. Number of connections involving IL-17A were compared in a prospective study which investigated determinants of mortality in hospitalized patients with HIV-1-associated TB.

References

1. WHO. Global Tuberculosis Report. 2019/2020. < <https://www.who.int/tb/global-report-2019-2020> >, (Accessed 12/18/2021).
2. Gupta A, Wood R, Kaplan R, Bekker LG, Lawn SD. Tuberculosis incidence rates during 8 years of follow-up of an antiretroviral treatment cohort in South Africa: comparison with rates in the community. PLoS One. 2012;7(3):e34156.
3. Rockwood N, Costa DL, Amaral EP, et al. *Mycobacterium tuberculosis* Induction of Heme Oxygenase-1 Expression Is Dependent on Oxidative Stress and Reflects Treatment Outcomes. Front Immunol. 2017;8:542.
4. Miranda P, Gil-Santana L, Oliveira MG, et al. Sustained elevated levels of C-reactive protein and ferritin in pulmonary tuberculosis patients remaining culture positive upon treatment initiation. PLoS One. 2017;12(4):e0175278.
5. Mesquita ED, Gil-Santana L, Ramalho D, et al. Associations between systemic inflammation, mycobacterial loads in sputum and radiological improvement after treatment initiation in pulmonary TB patients from Brazil: a prospective cohort study. BMC Infect Dis. 2016;16:368.
6. Andrade BB, Pavan Kumar N, Amaral EP, et al. Heme Oxygenase-1 Regulation of Matrix Metalloproteinase-1 Expression Underlies Distinct Disease Profiles in Tuberculosis. J Immunol. 2015;195(6):2763-73.
7. Oliveira-de-Souza D, Vinhaes CL, Arriaga MB, et al. Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations. Sci Rep. 2019;9(1):8002.
8. Vinhaes CL, Oliveira-de-Souza D, Silveira-Mattos PS, et al. Changes in inflammatory protein and lipid mediator profiles persist after antitubercular treatment of pulmonary and extrapulmonary tuberculosis: A prospective cohort study. Cytokine. 2019;123:154759.
9. Rockwood N, Sirgel F, Streicher E, Warren R, Meintjes G, Wilkinson RJ. Low Frequency of Acquired Isoniazid and Rifampicin Resistance in Rifampicin-Susceptible Pulmonary Tuberculosis in a Setting of High HIV-1 Infection and Tuberculosis Coprevalence. J Infect Dis. 2017;216(6):632-40.
10. Schutz C, Barr D, Andrade BB, et al. Clinical, microbiologic, and immunologic determinants of mortality in hospitalized patients with HIV-associated tuberculosis: A prospective cohort study. PLoS Med. 2019;16(7):e1002840.
11. Mayer-Barber KD, Andrade BB, Oland SD, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature. 2014;511(7507):99-103.
12. Ernst JD. The immunological life cycle of tuberculosis. Nat Rev Immunol. 2012;12(8):581-91.
13. Friedman N, Goldszmidt M, Wyner A. Data analysis with bayesian networks: a bootstrap approach. Proceedings of the Fifteenth conference on Uncertainty in artificial intelligence; Stockholm, Sweden. 2073819: Morgan Kaufmann Publishers Inc.; 1999. p. 196-205.
14. Prada-Medina CA, Fukutani KF, Pavan Kumar N, et al. Systems Immunology of Diabetes-Tuberculosis Comorbidity Reveals Signatures of Disease Complications. Sci Rep. 2017;7(1):1999.
15. Tsang J, Chain BM, Miller RF, et al. HIV-1 infection of macrophages is dependent on evasion of innate immune cellular activation. AIDS. 2009;23(17):2255-63.
16. Maddocks S, Scandurra GM, Nourse C, et al. Gene expression in HIV-1/*Mycobacterium tuberculosis* co-infected macrophages is dominated by *M. tuberculosis*. Tuberculosis (Edinb). 2009;89(4):285-93.
17. Rasaiyaah J, Tan CP, Fletcher AJ, et al. HIV-1 evades innate immune recognition through specific cofactor recruitment. Nature. 2013;503(7476):402-5.
18. McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 Family of Cytokines in Health and Disease. Immunity. 2019;50(4):892-906.

19. Veldhoen M. Interleukin 17 is a chief orchestrator of immunity. *Nat Immunol.* 2017;**18**(6):612-21.
20. Okamoto Yoshida Y, Umemura M, Yahagi A, et al. Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. *J Immunol.* 2010;**184**(8):4414-22.
21. Freches D, Korf H, Denis O, Havaux X, Huygen K, Romano M. Mice genetically inactivated in interleukin-17A receptor are defective in long-term control of *Mycobacterium tuberculosis* infection. *Immunology.* 2013;**140**(2):220-31.
22. Singh S, Maniakis-Grivas G, Singh UK, et al. Interleukin-17 regulates matrix metalloproteinase activity in human pulmonary tuberculosis. *J Pathol.* 2018;**244**(3):311-22.
23. Jasenosky LD, Scriba TJ, Hanekom WA, Goldfeld AE. T cells and adaptive immunity to *Mycobacterium tuberculosis* in humans. *Immunol Rev.* 2015;**264**(1):74-87.
24. Johnson TP, Patel K, Johnson KR, et al. Induction of IL-17 and nonclassical T-cell activation by HIV-Tat protein. *Proc Natl Acad Sci U S A.* 2013;**110**(33):13588-93.
- ~~25. Christensen-Quick A, Lafferty M, Sun L, Marchionni L, DeVico A, Garzino-Demo A. Human Th17 Cells Lack HIV-Inhibitory RNases and Are Highly Permissive to Productive HIV Infection. J Virol.~~
- ~~2016;**90**(17):7833-47.~~
2625. Veazey RS. Intestinal CD4 Depletion in HIV / SIV Infection. *Curr Immunol Rev.* 2019;**15**(1):76-91.
2726. Korb VC, Phulukdaree A, Lalloo UG, Chuturgoon AA, Moodley D. TB/HIV pleurisy reduces Th17 lymphocyte proportion independent of the cytokine microenvironment. *Tuberculosis (Edinb).* 2016;**99**:92-9.
2827. Noack M, Beringer A, Miossec P. Additive or Synergistic Interactions Between IL-17A or IL-17F and TNF or IL-1beta Depend on the Cell Type. *Front Immunol.* 2019;**10**:1726.
2928. Katz Y, Nativ O, Beer Y. Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a "fine-tuning cytokine" in inflammation processes. *Arthritis Rheum.* 2001;**44**(9):2176-84.
3029. Jovanovic DV, Di Battista JA, Martel-Pelletier J, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol.* 1998;**160**(7):3513-21.
3430. Elkington P, Tebruegge M, Mansour S. Tuberculosis: An Infection-Initiated Autoimmune Disease? *Trends Immunol.* 2016;**37**(12):815-8.

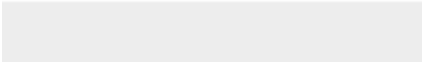
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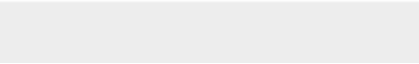


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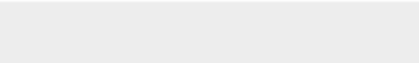


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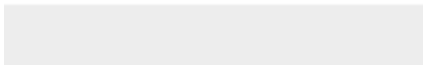


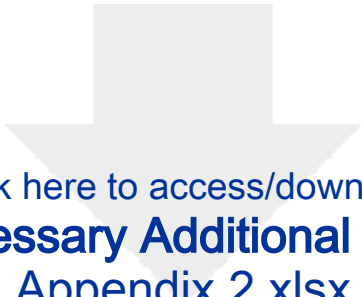


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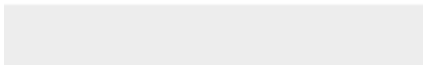


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Elsa du Bruyn, Neesha Rockwood, Charlotte Schutz, Katalin A. Wilkinson and Bruno B. Andrade performed experiments; Elsa du Bruyn, Neesha Rockwood, Charlotte Schutz, Katalin A. Wilkinson, Graeme Meintjes, Catherine Riou, Robert J Wilkinson and Bruno B. Andrade designed experiments; Elsa du Bruyn, Neesha Rockwood, Kiyoshi F. Fukutani, Catherine Riou and Bruno B. Andrade analyzed the data; Neesha Rockwood and Charlotte Schutz conducted the clinical recruitment, administered the projects and curated the respective datasets; Katalin A. Wilkinson, Graeme Meintjes and Robert J Wilkinson provided materials and infrastructural support; Elsa du Bruyn, Kiyoshi F. Fukutani, Neesha Rockwood, Juan M. Cubillos-Angulo, Maria B. Arriaga, Rafael Tibúrcio, Catherine Riou, Katalin A. Wilkinson, Charlotte Schutz, Graeme Meintjes, Alan Sher, Robert J Wilkinson and Bruno B. Andrade wrote the manuscript.

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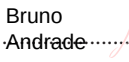
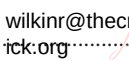
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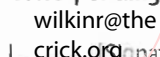
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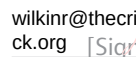
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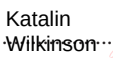
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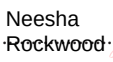
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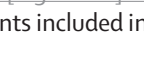
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