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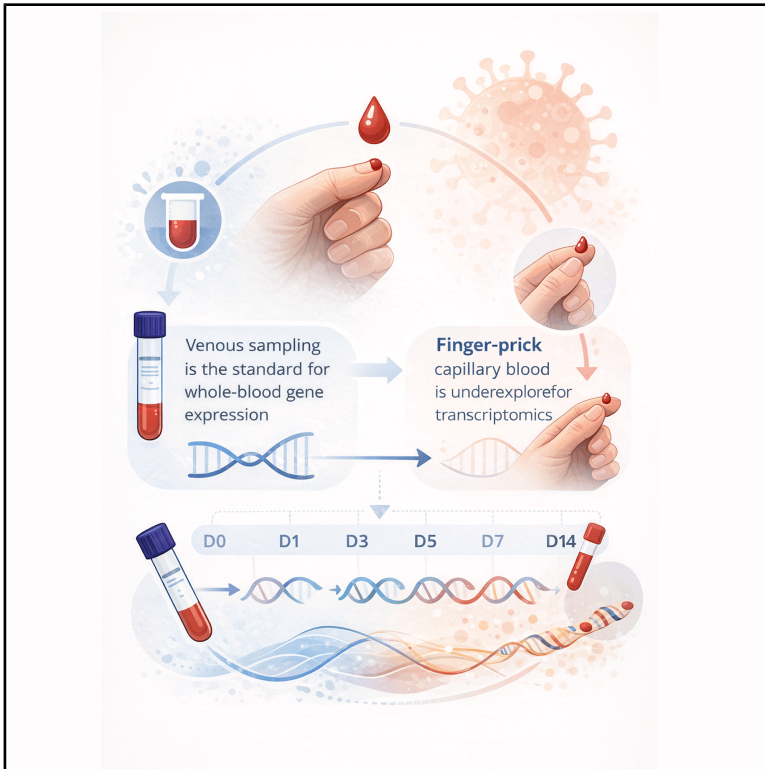
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Self-collected finger-prick blood for gene expression profiling: Unveiling early immune responses in mild COVID-19

Graphical abstract



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In brief

Biotechnology; Applied sciences

Highlights

- Finger-prick sampling enables at-home whole-blood transcriptomics in mild COVID-19
- Venous and capillary blood show moderate gene-level and excellent gene-set agreement
- High-frequency sampling reveals early interferon, neutrophil, and erythroid dynamics
- Finger-prick transcriptomics enables daily immune monitoring in outpatients



Article

Self-collected finger-prick blood for gene expression profiling: Unveiling early immune responses in mild COVID-19

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SUMMARY

Whole-blood gene expression analysis is essential for understanding molecular host responses, yet its use typically relies on venous sampling, limiting feasibility for frequent and remote monitoring. In an ancillary study of the COVERAGE France platform trial (NCT04356495), which enrolled at-risk outpatients with mild coronavirus disease 2019 (COVID-19) monitored at home, we compared transcriptomic profiles obtained from paired venous blood (Tempus tubes) and ultralow-volume, self-collected finger-prick capillary samples. We observed moderate to good concordance at the individual gene level and excellent agreement at the gene-set level between the two sampling approaches. High-frequency finger-prick sampling enabled daily resolution of immune dynamics, revealing early interferon responses, sustained neutrophil activation, and evolving erythroid and inflammatory signatures during the initial phase of mild COVID-19. These results demonstrate that finger-prick sampling is a feasible and reliable approach for at-home transcriptomic profiling, offering a powerful tool for longitudinal immune monitoring and advanced clinical research.

INTRODUCTION

At the era of systems biology and targeted therapies, analysis of whole-blood gene changes provides key information about molecular host responses to diseases, infections, or therapeutic interventions, and potentially markers or correlates of disease severity.^{1,2} However, full use of these approaches requires repeated sampling, especially around interventions such as vaccine injections or disease onset. Due to practical constraints, venous blood sampling is not feasible at high frequency or by the participants themselves. This issue becomes even more pronounced in certain groups, such as healthy volunteers, children, or in settings such as ambulatory care. Therefore, self-collected finger-prick capillary blood sampling offers a suitable alternative for performing frequent measurements.³ However, evaluations of such sampling remain limited. While microsampling tech-

niques have demonstrated promising results for proteomics quantifications,⁴ transcriptomics analyses on micro-samples are less common. It is thus important to determine whether venous and capillary samples yield comparable and reproducible results.

Several studies have compared changes in gene expression using capillary and venous blood samples, with discordant reported findings.^{5,6} However, more promising results have been presented elsewhere in terms of RNA quality,⁷ agreement between finger-prick and venipuncture,⁸ as well as clinical predictive capacity.⁹ An ultralow-volume sampling procedure for self-collection and subsequent RNA preservation of a few drops of blood (50 μ L) collected by finger prick has been successfully used to explore the response to coronavirus disease 2019 (COVID-19) vaccine.^{10,11} In the context of COVID-19, a large amount of data has been generated during hospitalization¹²



or in the post-hospitalization period,^{13,14} but there are limited studies available examining the early stages of immune response in non-hospitalized patients.^{9,15–17}

In the present work, we directly compared data derived from blood samples obtained simultaneously by venous puncture and ultralow-volume sampling collected by finger prick, allowing the quantification of whole-blood gene abundance, applied to a cohort of patients with COVID-19 followed up at home in the framework of a clinical trial. We correlated changes in gene abundance with whole-blood cell phenotype and serum biomarkers collected by venous puncture at fixed time points. Our results validate the finger-prick procedure, its feasibility at participant's home, and provide insights about the dynamics of gene-abundance changes during the early phase of mild COVID-19 infection.

RESULTS

Participants and study feasibility

The COVERAGE France platform trial was designed to evaluate various treatment strategies in non-hospitalized patients with documented early COVID-19 and risk factors for aggravation of documented COVID-19. Patients with symptoms lasting ≤ 7 days were recruited into the trial. The protocol has already been described.^{18,19} An ancillary study was designed to explore host response in a subset of volunteering participants. After initial training in the self-sampling method, the following sampling schedule was applied in volunteers: i) a venous puncture was performed by a nurse at the participants' home on D0, D7, and D14, and was used for flow cytometry cell phenotyping, quantification of serum analytes, and RNA gene expression from whole blood; ii) finger-prick self-sampling of capillary blood was performed at home by the participants themselves after education on D0, D1, D3, D5, D7, D9, D12, and D14, and was used for the characterization of RNA gene expression (Figure S1).

This ancillary study enrolled twenty-two participants from the COVERAGE trial recruited at the Bordeaux site (mean age, 65 years; 8 females) from August to November 2020. In median, patients were enrolled 4 (interquartile range [IQR], 3–6) days after symptoms onset. They were randomized to a control arm (no treatment, $n = 10$) or to intervention arms (imatinib; $n = 3$; telmisartan, $n = 9$) (Table S1). Two participants were subsequently hospitalized (at D5 and 10, respectively); none was admitted to an intensive care unit, and none died during the 14 days of follow-up after inclusion. A total of 156 finger-prick capillary samples were performed and analyzed, with 130/156 (83%) showing a good RNA integrity number (RIN) > 6 (Figure S2), demonstrating the feasibility of intensive self-sampling at home despite acute sickness.

Overview of early cell phenotype and serum biomarker profiles in patients with mild COVID-19

Cell phenotyping analysis showed an increase in the frequency of naive CD4⁺ T cells at D7, which contrasts with the decrease of this cell population reported in more advanced patients.^{15,20} At D14, we observed an increase in the expression of the activation marker HLA DR + on CD4⁺ and CD8⁺ T cells, as previously reported in patients with mild to severe disease by our group

and others.^{21–24} In addition, we observed an increase in the proportion of myeloid dendritic cells (mDC) without a decrease in plasmacytoid dendritic cells (pDC), in contrast to more advanced patients²³ (Figure 1A).

Measurement of serum markers revealed a low level of inflammation in these patients, attested by the lack of elevation of markers such as IL-8 and TNF, described in patients with severe COVID-19.^{25,26} Similarly, interferon gamma-induced protein 10 (IP10), IL-1b, phospholipase D1 (PDL1), IL18, and Fas ligand, present at D0, decreased significantly at D14, exhibiting a profile of a transient burst, as previously described in people with mild infection⁹ and which differs from severe infections. Of note, interferon (IFN) levels remained stable from baseline (Figure 1B). Therefore, patients enrolled in the COVERAGE ancillary study exhibited clinically and biological profiles consistent with mild COVID-19¹⁵ (Figure S3). In this context, we sought to extend investigations of the dynamics of early molecular immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by conducting a thorough analysis of additional markers measured more frequently, leveraging the gene expression abundance data obtained from finger-prick capillary samples.

Comparison between finger-prick and venous sampling demonstrated good concordance and pathways enrichment

First, we compared the abundance of gene expression at D7 and D14 with D0 in samples collected by finger prick or venous blood puncture. The intraclass correlation of gene abundance between the two sampling methods was good ($r = 0.74$, Figure 2A). In the Bland and Altman plot (average of the two measures on the x axis and difference between the two measures on the y axis), the limits of agreement (2.78 and -2.98 log₂ count per million (CPMs)) were calculated as 8 CPMs on the natural scale, hence being less than the usual thresholds (10, 40) defining the minimum level to consider a count interpretable (Figure 2B).

Then, we looked at the differential abundance of genes between D0 and D7 and D14 provided by the two techniques using the dearseq methods²⁷ (Figure S4). At D7, 265 and 223 genes were differentially abundant in finger-prick and venous blood samples, respectively. The number of differentially abundant genes increased at D14, reaching 352 and 690 genes in finger-prick and venous blood samples, respectively. Among the top 20 genes differentially expressed at D7, 10 were similar regardless of the sampling method: *HESX1*, *OTOF*, *LAMP3*, *PI3*, *CCL8*, *CCL2*, *TMEM255A*, *SIGLEC1*, *IFI44L*, and *RSAD2* (Figure S4). Interestingly, among these top 20 genes, *RSAD2* (viperin) and *IFI44L* are IFN-stimulated genes with strong antiviral activity. Besides, *CCL8* (MCP-2) and *CCL2* (MCP-1) are monocyte chemoattractant cytokines that are significantly stimulated during COVID-19 infection. At D14, although the signal seemed lower in the finger-prick samples, 11 of the top 20 genes were common to the two sampling methods (*KCTD14*, *HESX1*, *OTOF*, *IFI27*, *SIGLEC1*, *LAMP3*, *IFI44L*, *RSAD2*, *ZNF496-DT*, *LY6E-DT*, and *SPP1*). Among the top 20 genes from finger-prick samples at D7 and D14, 12 and 19 genes, respectively, were also identified as differentially expressed in venous samples.

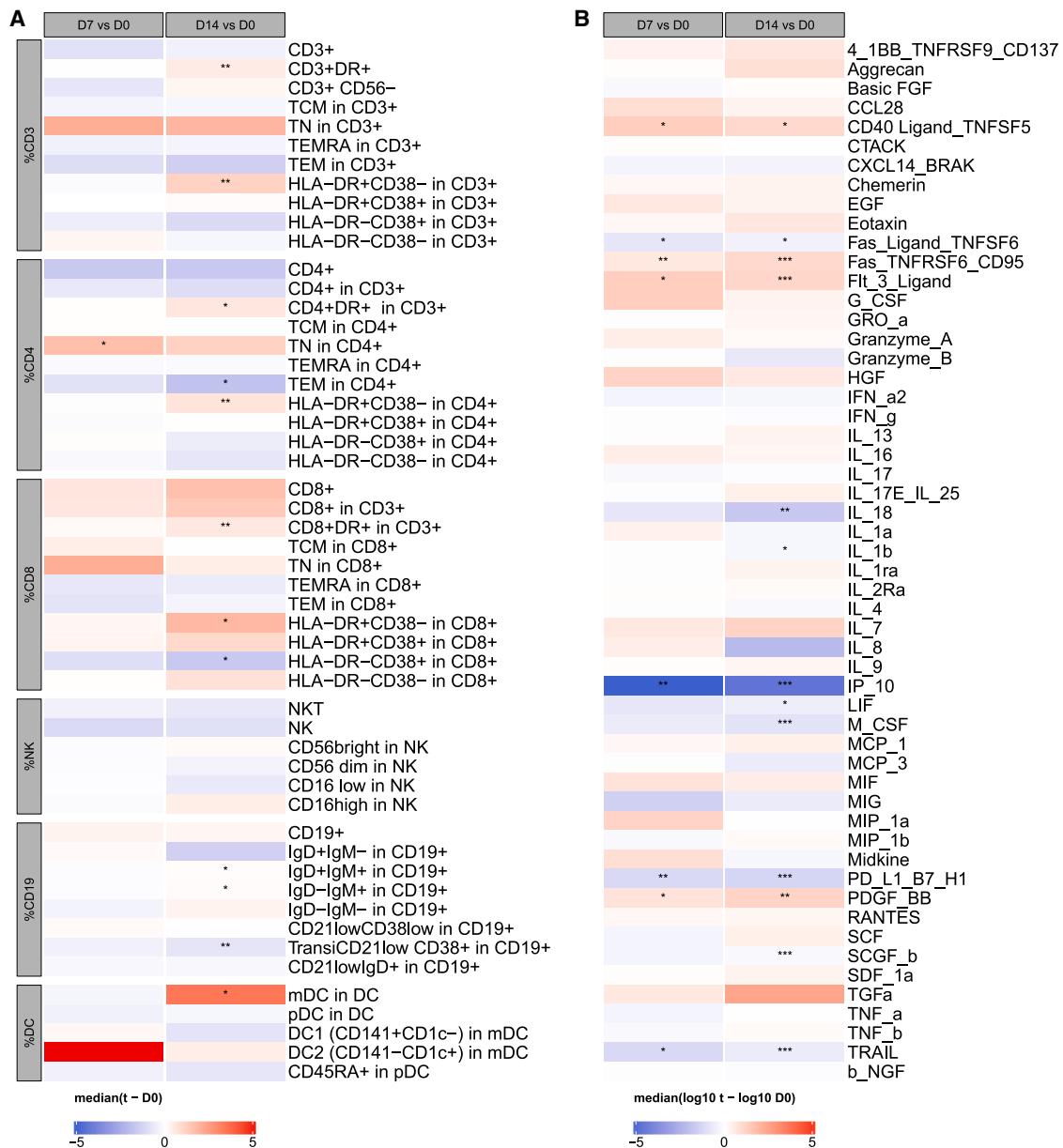


Figure 1. Cellular and serum cytokine profiles over time

(A). Heatmap showing the median differences in immune cell subset proportions at day 7 (D7) and day 14 (D14) compared with baseline (day 0). Each row corresponds to a cell type, and colors indicate relative increases (red) or decreases (blue).

(B). Heatmap showing the median log10 fold-change in serum cytokine levels at D7 and D14 relative to baseline. Each row corresponds to a cytokine type, and colors indicate relative increases (red) or decreases (blue). Statistical comparisons were performed using the Wilcoxon signed-rank test with false discovery rate (FDR) correction. Significance thresholds are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

To confirm the interest of the finger-prick approach with regard to the moderate to good agreement between the two techniques at the gene level, we extended the comparison of the results between the two sampling methods in terms of gene-set enrichment (Figure 3), which are groups of genes defined *a priori* using other datasets.²⁸ We hypothesized that this comparison would be more relevant, as it is less sensitive to variation in signal at the gene level. Analyses were carried out using a fixed reper-

toire of 382 transcriptional modules (BloodGen3) that are functionally characterized.²⁹ The gene composition of the modules and their functional annotations are provided at <https://drinchai.shinyapps.io/BloodGen3Module/>. “Module aggregate” are sets of modules that showed consistent abundance profiles across a reference set of 16 disease cohorts used for the construction of the BloodGen3 repertoire.²⁹ Although more modules appeared to show changes in abundance over time with the

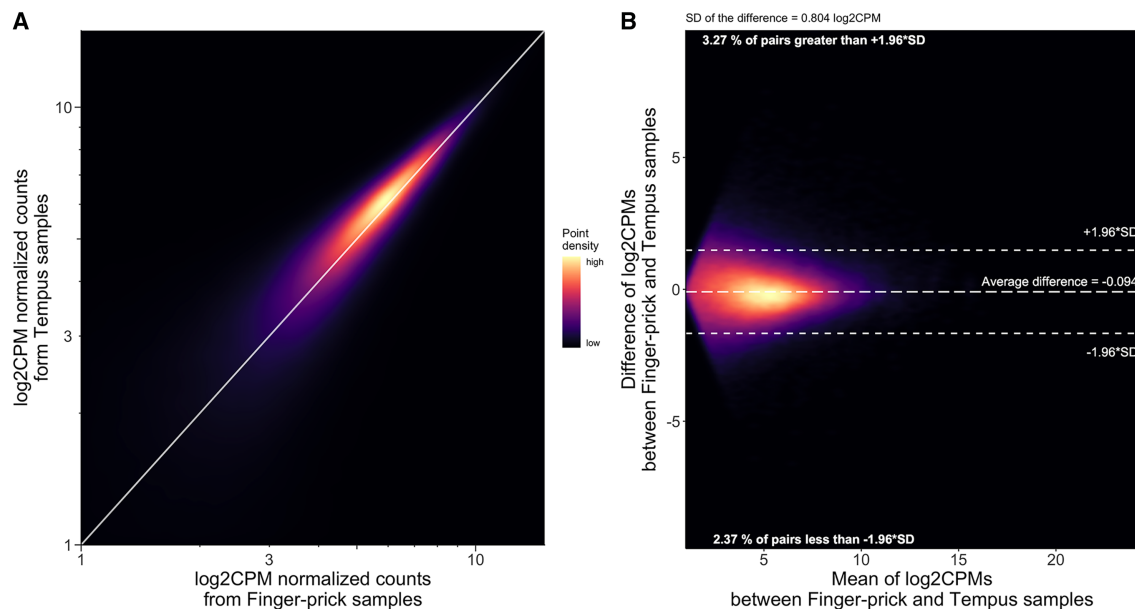


Figure 2. Agreement between the RNA sequencing normalized data of COVID-19+ outpatients measured by finger-prick and Tempus sampling (all genes, all time points)

(A). Scatterplot showing the correlation between normalized RNA sequencing data obtained from finger-prick samples and matched venous Tempus tube samples across all genes and time points. The overall correlation coefficient was $r = 0.74$, indicating moderate agreement.

(B). Bland-Altman plot assessing agreement between the two sampling methods. The x axis represents the average of finger-prick and Tempus values, while the y axis shows the difference between paired measurements. Limits of agreement are displayed, with 93% of observations falling within the range. These panels illustrate that, while individual gene-level agreement is moderate, overall transcriptomic profiles between finger-prick and venous blood are consistent.

venous blood sampling methods compared with the finger-prick approach (Figure 3), the agreement between the methods was excellent: the intraclass correlation coefficients of the average fold change between modules at D7 and D14 were $r = 0.89$ and $r = 0.97$, respectively (Figure S11). The abundance of genes from module M10.4 (*BPI*, *CEACAM6*, *CEACAM8*, *CTSG*, *DEFA1*, *DEFA3*, *DEFA4*, *ELA2*, *LOC653600*, *LOC728358*, *LTF*, *MPO*, and *OLFM4*), annotated “neutrophils activation,” was found to be significantly increased at day 7 and day 14 with both sampling methods. The abundance of five of the six modules belonging to the module aggregate A28, annotated “interferon,” was consistently decreased with both sampling methods at both time points, as was the module “Type 1 IFN,” including *IFI*, *IFIT*, *OAS*, *EPST11*, *HERC5*, *HES4*, *IFI44*, *IFI44L*, *IFI6*, *IFIT1*, *IFIT3*, *ISG15*, *LY6E*, *MX1*, *OAS1*, *OAS2*, *OAS3*, *OASL*, *RSAD2*, and *XAF1* genes (Figures 5 and 6). The aggregate annotated as “erythroid cells” presented an increased abundance at D14 only, but consistently with both sampling methods. Consistently, both sampling methods, albeit with a weaker signal, also showed the modules “cell cycle,” “inflammation,” “protein synthesis,” and “monocytes” as differentially abundant at D7 and D14 compared with D0.

These results showed high consistency in the enrichment of gene sets between the two sampling methods and confirmed signals such as neutrophils activation, IFN signaling, erythroid cells, and inflammation pathways at the early stages of mild COVID-19. Several of these changes are in agreement with those previously described and associated with severe disease by our group and others.³⁰ However, these changes were described at

later time points after disease onset (1 month) and persisted for several months in the setting of long COVID.³⁰ Therefore, while the persistence of these abnormalities is well described in hospitalized patients with severe disease, the dynamics of these immune disturbances during the early stages of infection in outpatients are not fully described.

The high-temporal-resolution transcriptomic analysis using finger-prick sampling revealed a more precise dynamics of gene abundance from key pathways.

As described above, the dynamics of changes in gene abundance were initially limited to the resolution of only two time points (D7 and D14) because of the constraints of venous blood sampling. Finger-prick sampling allowed us to better decipher early gene changes every 2 days from D0 to D14. We showed that patients with mild COVID-19 experienced an IFN response, with an increase in related gene abundance from D1 and a rapid decrease from D3 to D9 (Figures 4A and 4B; S7A and S7B). In contrast, neutrophil activation, attested by increased abundance of genes from the neutrophil module, increased from D1 and persisted until D12 (Figures 4C and 4D; S7C and S7D). With regard to aggregate A37 (“erythroid cells”), the dynamics of gene abundance were very different, with an increase starting on D3 through D14 (Figures 4E and 4F; S7E and S7F).

Overall, we show here that repeated finger-prick sampling revealed a more complex and non-linear dynamics in changes in gene abundance of critical pathways at the early stages of mild COVID-19. For instance, we showed that an early IFN response may have been beneficial, which is consistent with the outcomes in these patients.²⁸ This response waned within a few days

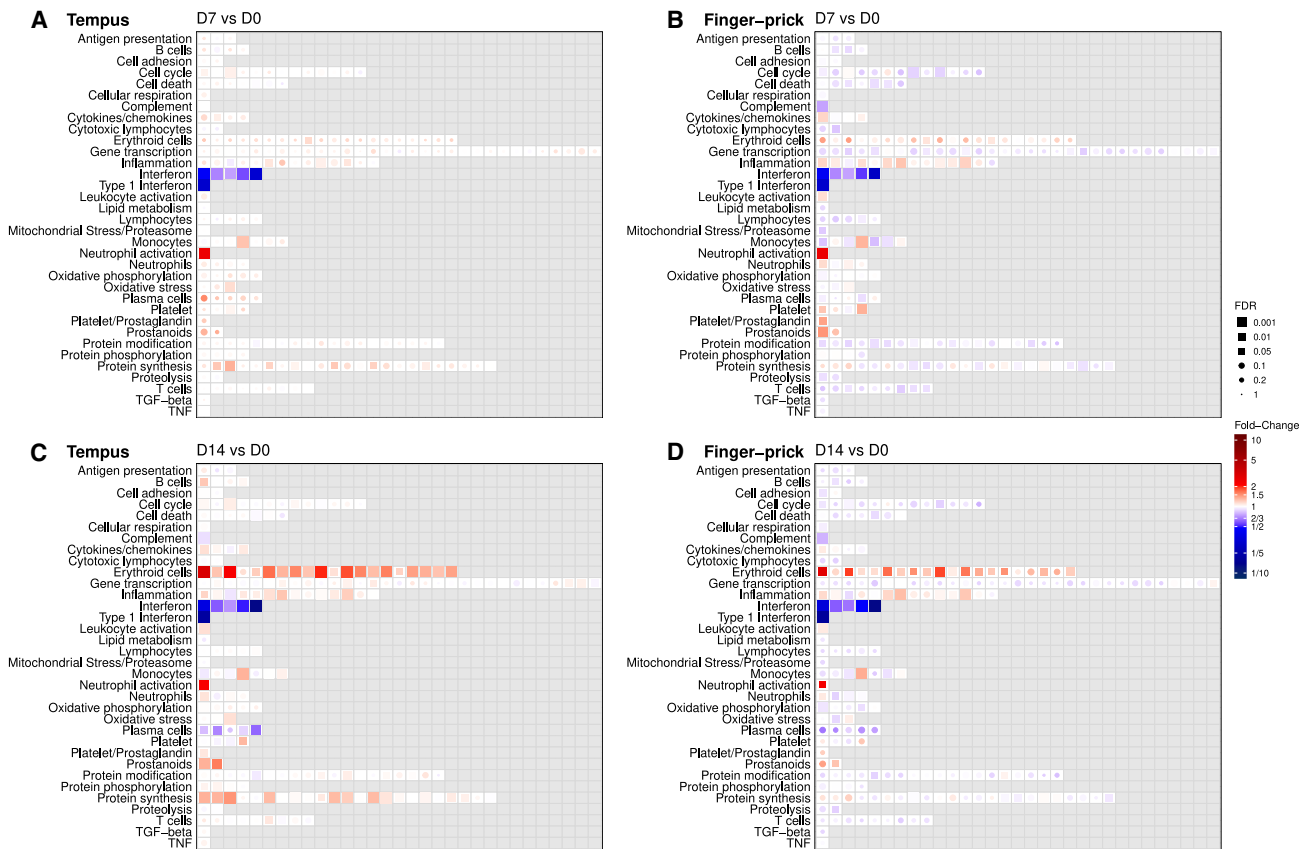


Figure 3. Longitudinal gene set enrichment in venous and finger-prick samples

Description of enriched gene sets at day 7 post-inclusion measured in venous blood and finger-prick samples. Enriched gene sets are shown at day 7 (A, B) and day 14 (C, D) in the 22 COVID-19+ outpatients of the COVERAGE immune study.

Colored modules indicate significant differential abundance relative to baseline, defined as an absolute fold change (FC) > 1.5. Squares indicate a false discovery rate (FDR) < 0.05, while circles indicate higher FDRs. Color intensity reflects the magnitude and direction of change, with red representing up-regulation and blue representing down-regulation (based on median fold change). This analysis highlights concordant immune pathways captured by both venous and finger-prick sampling methods, including modules associated with interferon signaling, neutrophil activation, and, albeit with a weaker signal, the modules “cell cycle,” “inflammation,” “protein synthesis,” and “monocytes.” Each non-grey square represents a module, and each line represents a shared annotation (e.g., 1 module is annotated with “neutrophil activation,” while 5 modules are annotated with “Interferon” — see Figure S6B for additional information).

(beyond day 3), indicating that studies examining later time points may miss these early changes in gene expression,^{31,32} with the exception of human challenge studies that reported similar results.¹⁷ Globally, repeated at-home measurements allowed linking a favorable clinical evolution of COVID-19 in elderly patients to a quick resolution, within a couple of days, of the immune response to infection.

Correlation between whole-blood gene expression and variations in cell populations and cytokine levels

Several deconvolution algorithms have been proposed to infer blood transcriptomic changes in relation to variations in various type of cells.^{22,33,34} Here, we used the CIBERSORTx³⁵ algorithm with the TIL10³⁶ reference matrix to predict the abundance of several cell types, and we compared these predictions based on genes abundance with the actual proportions measured by flow cytometry. We observed very modest agreement between predictions based on gene abundance and the frequency of cells as assessed by flow cytometry (concordance

correlation coefficient [CCC] = 0.50 and 0.57 from Tempus; 0.47 and 0.50 from finger prick, for B cells and CD8+ T cells, respectively; Figures 5A and 5B, respectively) and even lower agreement for CD4+ T cells, natural killer (NK) cells, or neutrophils (CCC between 0.05 and 0.40; Figures 5C–5E), but still in the same order between the two sampling methods. Hence, regardless of the quality of the prediction of cell abundance, the two sampling methods produced close results. More interestingly, we examined the concordance of dynamics between gene expression and two markers of inflammation, namely, IL-1b and IP-10 (Figure 6A). We observed high precision in the temporal trends of these markers when measured by Luminex, with correlations for IP-10 levels of 0.70 and 0.68 between Luminex and gene expression from Tempus and finger-prick samples, respectively. Moreover, the two-day finger-prick sampling allowed a detailed analysis of these dynamics, showing increased and stable expression over the first 3 days before declining in an approximately linear fashion over time (Figure 6B). The rapid decrease of IL1b observed is

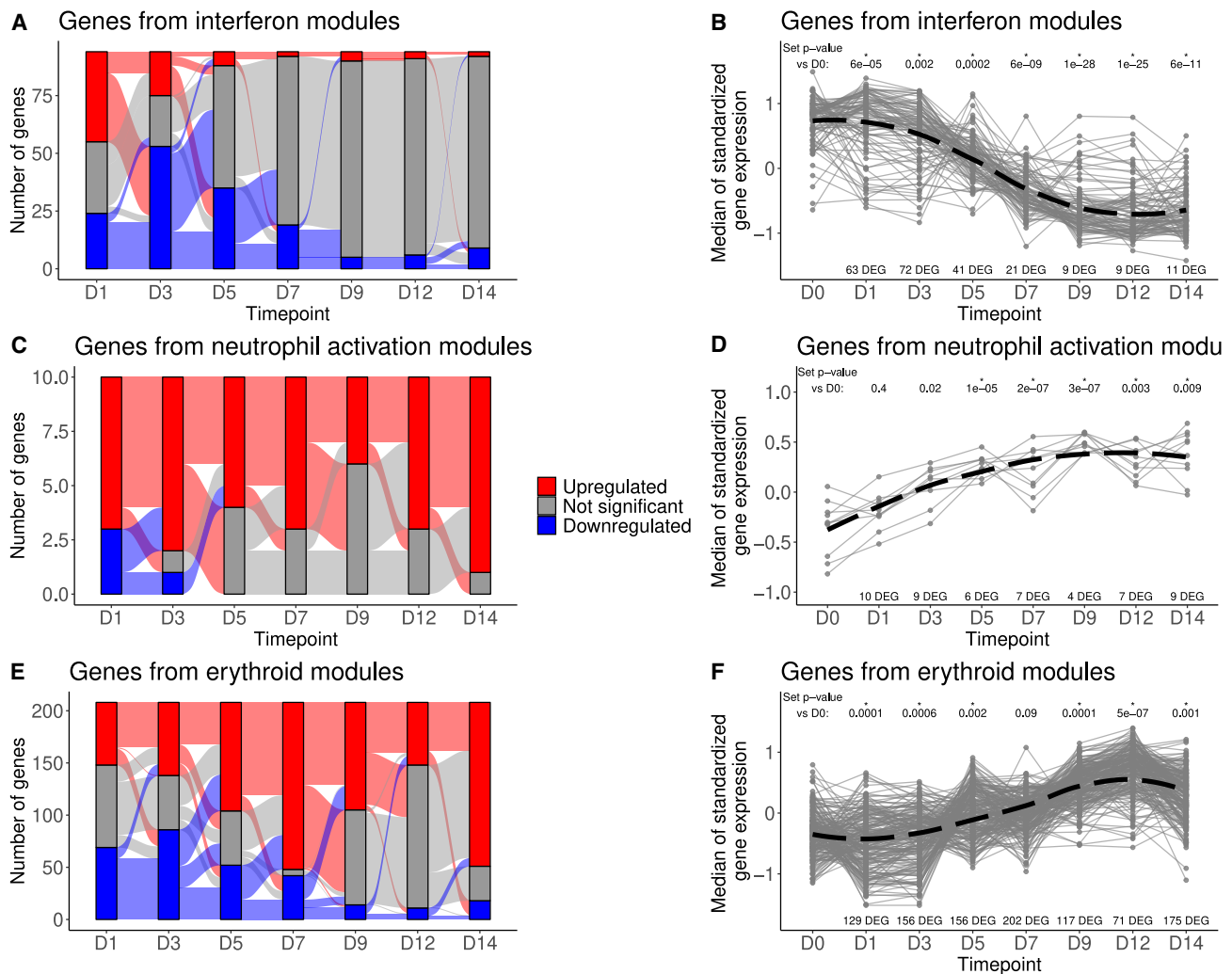


Figure 4. Longitudinal changes in gene abundance related to interferon regulation, neutrophil activation, and erythroid cells using finger-prick capillary sampling

Differential gene expression analysis was performed every 2 days using finger-prick capillary samples from COVID-19 outpatients. (A) and (B) show genes from interferon-related modules, (C) and (D) show genes from neutrophil activation modules, and (E) and (F) show genes from erythroid cell-associated modules. Each point represents the change in gene abundance at a given time point compared with baseline (day 0), illustrating the temporal dynamics of immune activation during early infection. (A), (C), and (E) are alluvial plots, representing the number of genes either significantly up-regulated, significantly down-regulated, or non-significant as stacked bar charts at each time point, as well as flow curves showing the number of genes switching categories from one time point to the next. (B), (D), and (F) represent each gene of the gene set over time as a line, using its median expression across all subjects. The heavy dotted line is a smoothed average of the median expression over all the genes from the displayed module. Represented *p*-values are computed by the dearseq gene set score test, comparing each time point to D0. The number of differentially expressed gene (DEG) indicated at each time point is identified from the gene-wise approach from dearseq. Using repeated finger-prick sampling, we were able to capture fine-grained changes in gene expression every 2 days from D0 to D14, providing a detailed view of early immune dynamics in mild COVID-19.

in agreement with the reduction of circulating inflammatory monocytes reported in human challenge studies.¹⁷

DISCUSSION

Overall, the comprehensive data generated in this study provide a novel approach to interrogate the molecular mechanisms underlying host responses at the early stages of COVID-19 in a population of patients at high risk of aggravation, defined by age and comorbidities. We showed that obtaining

gene expression data from a finger-prick has obvious advantages compared with relying on specific time points constrained by venous punctures. These new data exploring early responses to SARS-CoV-2 infection extend previous reports that successfully used finger-prick sampling to characterize autoimmunity,³⁷ as well as the response to infection³⁸ or to vaccination.³⁹ We showed here that this sampling approach could be useful to identify pathways and characterize the dynamics of gene expression that are missed by more targeted studies with predefined later time points. One interesting

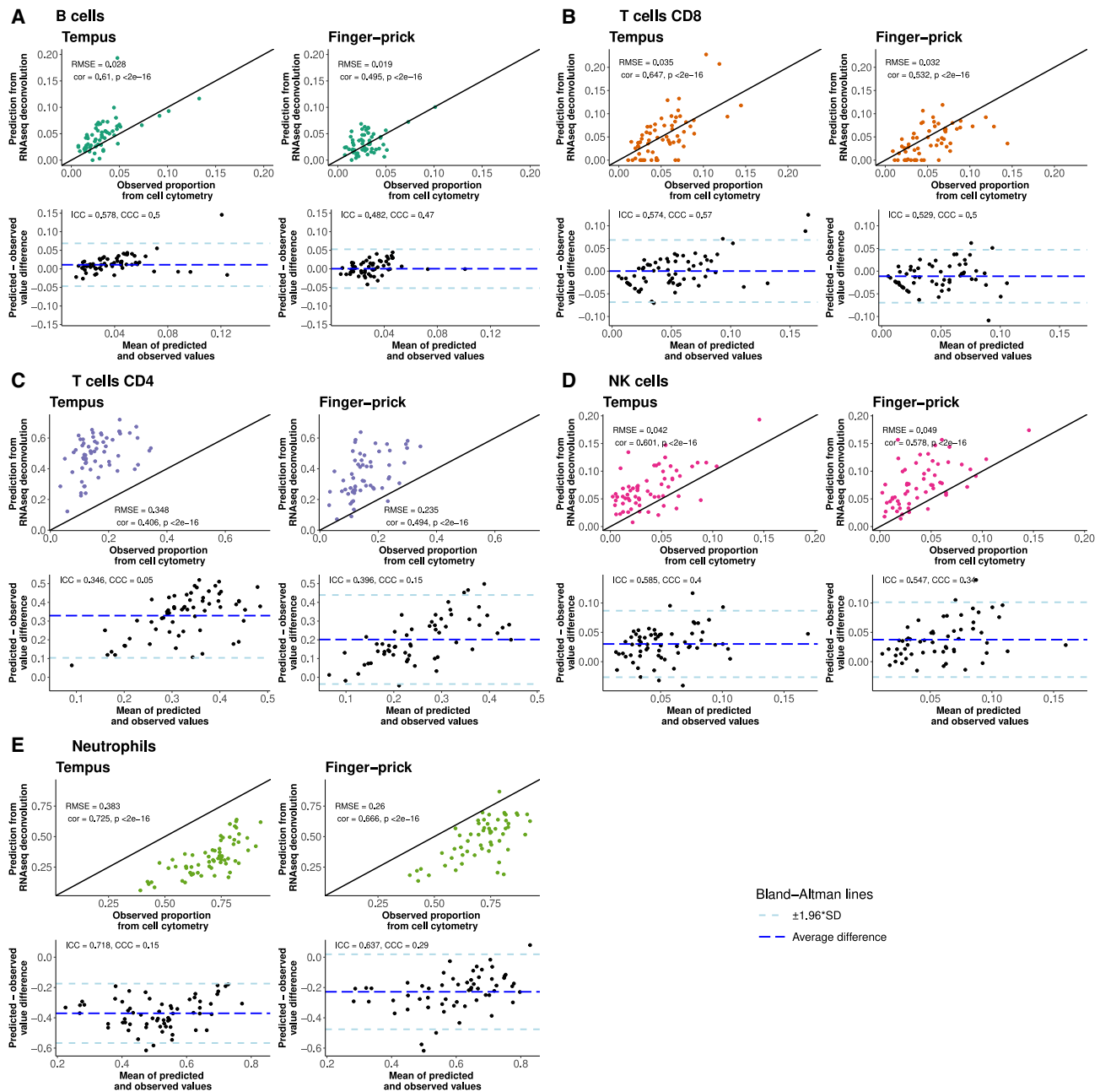


Figure 5. Correlation between cell-type-specific gene expression and immune cell abundance measured by flow cytometry

Scatterplots show the correlation between the abundance of cell-type-specific gene expression signatures measured by RNA sequencing from venous blood (Tempus, left) or finger-prick samples (right) and the corresponding immune cell frequencies measured by flow cytometry. Each point represents an individual paired measurement. Correlation coefficients (r), p -values, and root-mean-square error (RMSE) are shown on each plot. Bland-Altman plots assess agreement between the abundance of cells predicted from RNA sequencing and the corresponding immune cell frequencies measured by flow cytometry. The x axis represents the average of predicted and observed cell abundance values, while the y axis shows the difference between predicted and observed cell abundance values. Intraclass correlation coefficients (ICC) and concordance correlation coefficient (CCC) are shown on each plot. Cell populations include A) B cells, B) CD8⁺ T cells, C) CD4⁺ T cells, D) neutrophils, and E) NK cells. While inferring immune cell composition from bulk RNA-seq data from blood remains very challenging, finger-prick samples do not present a reduced capacity for cellular deconvolution compared with Tempus samples.

information provided by this study, thanks to repeated measurements, is that the earliest stages of mild SARS-CoV-2 infection are characterized by the same pathways described

in patients with severe disease, but with a rapid resolution. In addition, we showed correlations between gene abundance and deconvolution of certain cell frequencies, as well as serum

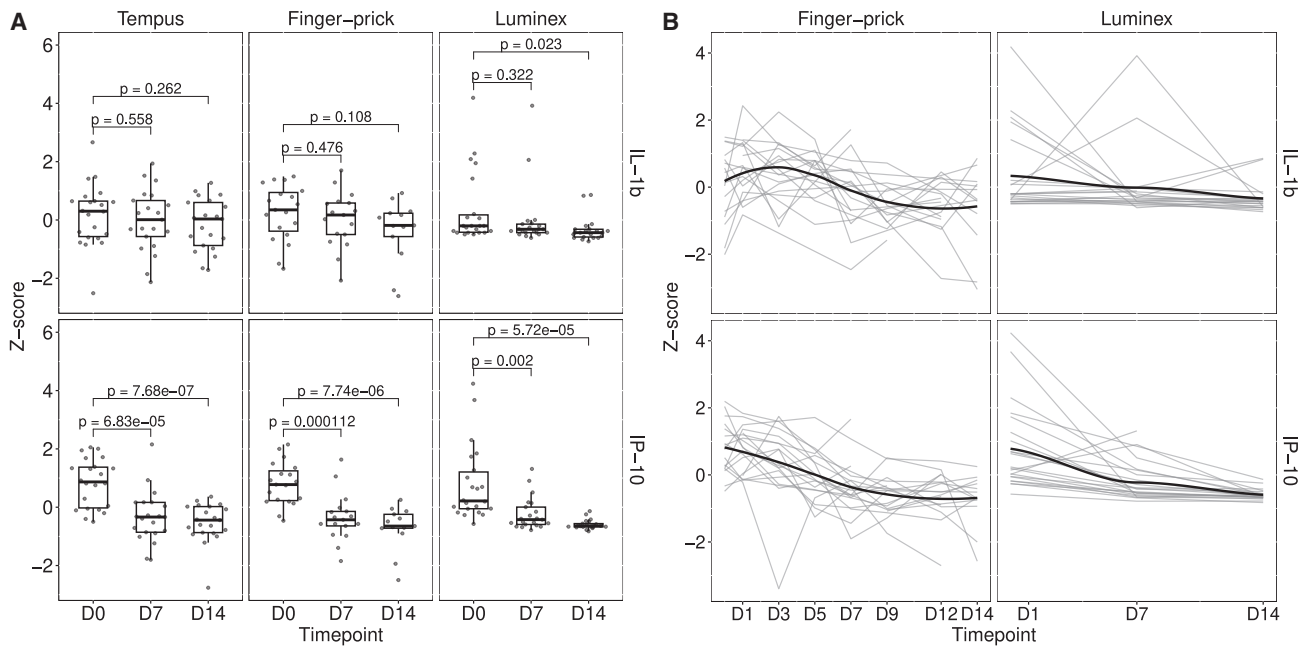


Figure 6. Longitudinal analysis of inflammatory markers (IL-1 β , IP-10) measured by RNA sequencing (Tempus and finger-prick) and Luminex (serum)

(A) Boxplots showing IL-1 β and IP-10 abundance (Z scores) at day 0 (D0), day 7 (D7), and day 14 (D14), measured using three approaches: RNA sequencing from venous blood (Tempus), RNA sequencing from finger-prick samples, and serum protein quantification by Luminex.

(B) Longitudinal trajectories of IL-1 β and IP-10 measured by finger-prick RNA sequencing and serum Luminex. Gray lines represent individual patient trajectories, and the black line represents the median. Together, these data show that temporal trends in inflammatory marker expression detected by finger-prick sampling closely mirror those observed by venous RNA sequencing and protein-level dynamics measured by Luminex.

biomarker dynamics, which represent an opportunity for clinical research and for better deciphering the physiopathology of SARS-CoV-2.

These data provide a possible explanation for previous studies raising concerns about the utility of finger-prick gene expression analysis because of the reported lack of agreement with venous blood sampling.⁶ Although biological explanations could be hypothesized based on previously published differences in cell frequencies⁴⁰ or cytokines,⁴¹ a technical bias should be ruled out before implementing finger-prick analysis. For example, we re-analyzed previously published microarray data⁵ for which Stein et al.⁶ originally claimed there was a strong divergence between the two sampling techniques. We show instead that this discrepancy could be linked to a batch effect, which is absent in our present study. Therefore, our results reinforce the utility of the finger-prick method and also underscore the requirement to check for potential biases in sequence generation.

Finger-prick samples yield a smaller total RNA quantity compared with venous blood draws. Genes expressed at very low levels might fall below the reliable detection threshold in some finger-prick samples, even with sensitive mRNA-targeted library preparation. This can lead to variability, affecting correlation with venous samples, where these low-abundance transcripts might be more consistently captured, explaining the moderate agreement at the gene level. Confirmatory studies may require more precise measurements of targeted genes, using PCR, for instance.

Our study has limitations. One of those is the low number of participants. Despite this, we were able to demonstrate statistical concordance between the two sampling methods in the analysis of gene abundance. Another limitation is that samples were analyzed as singletons, which does not allow formal assessment of technical reproducibility or differentiation between intra-assay and inter-individual variability. This choice was driven by the study design, which aimed to evaluate the feasibility of at-home sampling under conditions similar to those of clinical trials, where samples are typically processed as singletons. The objective of our study was to demonstrate the feasibility of at-home sampling in the context of acute SARS-CoV-2 infection; we were not able to analyze the impact of therapeutic interventions (imatinib, telmisartan). We showed some early features of SARS-CoV-2 infection in elderly patients infected in 2020–2021, when the COVERAGE trial was launched. Whether these earliest molecular and immunological profiles remain relevant with subsequent variants remains to be investigated.

The use of finger-prick tests in clinical research represents a genuine opportunity to identify early biomarkers following a diagnosis or intervention, to conduct intensive studies of their dynamics, to identify the best timing for further analyses, and to do so with samples collected by the participants themselves.

Limitations of the study

This study has limitations. The sample size was relatively small, which may limit generalizability. Samples were analyzed as

singletons, preventing formal assessment of technical reproducibility and separation of technical from inter-individual variability; this reflects standard clinical trial conditions and the study's primary objective of assessing at-home sampling feasibility rather than analytical performance. The study was not designed to evaluate treatment effects from the parent trial. Finally, the findings reflect early SARS-CoV-2 infections in elderly patients enrolled in 2020–2021, and their relevance to subsequent viral variants remains to be determined.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rodolphe Thiébaud (Email: rodolphe.thiebaud@u-bordeaux.fr).

Materials availability

The study did not generate new unique reagents.

Data and code availability

- Data: Data supporting this study can be accessed in the BioStudies database - [ebi.ac.uk \(E-MTAB-15066\)](https://europe.ebi.ac.uk/E-MTAB-15066).
- Code: Code related to this publication is available at <https://doi.org/10.5281/zenodo.14447221>

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AUTHOR CONTRIBUTIONS

R.T., E.L., B.H., and Y.L. designed the study. X.A. and D.M. enrolled and followed the patients and recorded clinical data. H.H., M.S., I.P., and A.B.M. performed the biological analyses. M.H., B.H., M.P., E.L., and R.T. performed the statistical analyses. R.T., Y.L., and E.L. drafted the first version of the manuscript. All authors critically revised the manuscript for important intellectual content and approved the final version before submission. E.L. and H.H. contributed equally to this work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Davis, M.M., Tato, C.M., and Furman, D. (2017). Systems immunology: just getting started. *Nat. Immunol.* *18*, 725–732.
2. Arunachalam, P.S., Scott, M.K.D., Hagan, T., Li, C., Feng, Y., Wimmers, F., Grigoryan, L., Trisal, M., Edara, V.V., Lai, L., et al. (2021). Systems vaccinology of the BNT162b2 mRNA vaccine in humans. *Nature* *596*, 410–416.
3. Lei, B.U.W., and Prow, T.W. (2019). A review of microsampling techniques and their social impact. *Biomed. Microdevices* *21*, 81.
4. Shen, X., Kellogg, R., Panyard, D.J., Bararpour, N., Castillo, K.E., Lee-McMullen, B., Delfarah, A., Ubellacker, J., Ahadi, S., Rosenberg-Hasson, Y., et al. (2024). Multi-omics microsampling for the profiling of lifestyle-associated changes in health. *Nat. Biomed. Eng.* *8*, 11–29.
5. Obermoser, G., Presnell, S., Domico, K., Xu, H., Wang, Y., Anguiano, E., Thompson-Snipes, L., Ranganathan, R., Zeitner, B., Bjork, A., et al. (2013). Systems Scale Interactive Exploration Reveals Quantitative and Qualitative Differences in Response to Influenza and Pneumococcal Vaccines. *Immunity* *38*, 831–844.
6. Stein, D.F., O'Connor, D., Blohmke, C.J., Sadarangani, M., and Pollard, A.J. (2016). Gene expression profiles are different in venous and capillary blood: Implications for vaccine studies. *Vaccine* *34*, 5306–5313.
7. Lim, F.Y., Kim, S.Y., Kulkarni, K.N., Blazevic, R.L., Kimball, L.E., Lea, H.G., Haack, A.J., Gower, M.S., Stevens-Ayers, T., Starita, L.M., et al. (2023). Longitudinal home self-collection of capillary blood using homeRNA correlates interferon and innate viral defense pathways with SARS-CoV-2 viral clearance. Preprint at medRxiv. <https://doi.org/10.1101/2023.01.24.23284913>.
8. Robison, E.H., Mondala, T.S., Williams, A.R., Head, S.R., Salomon, D.R., and Kurian, S.M. (2009). Whole genome transcript profiling from fingerstick blood samples: a comparison and feasibility study. *BMC Genom.* *10*, 617.
9. Toma, R., Duval, N., Pelle, B., Parks, M.M., Gopu, V., Torres, P.J., Camacho, F.R., Shen, N., Krishnan, S., Hatch, A., et al. (2020). A clinically validated human capillary blood transcriptome test for global systems biology studies. *Biotechniques* *69*, 289–301.
10. Rinchai, D., Anguiano, E., Nguyen, P., and Chaussabel, D. (2016). Finger stick blood collection for gene expression profiling and storage of tempus blood RNA tubes. *F1000Res.* *5*, 1385.
11. Rinchai, D., Deola, S., Zoppoli, G., Kabeer, B.S.A., Taleb, S., Pavlovski, I., Maacha, S., Gentilcore, G., Toufiq, M., Mathew, L., et al. (2022).

- High-temporal resolution profiling reveals distinct immune trajectories following the first and second doses of COVID-19 mRNA vaccines. *Sci. Adv.* **8**, eabp9961.
12. Liu, J., Li, S., Liu, J., Liang, B., Wang, X., Wang, H., Li, W., Tong, Q., Yi, J., Zhao, L., et al. (2020). Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *eBioMedicine* **55**, 102763.
 13. Rodriguez, L., Pekkarinen, P.T., Lakshmikanth, T., Tan, Z., Consiglio, C.R., Pou, C., Chen, Y., Mugabo, C.H., Nguyen, N.A., Nowlan, K., et al. (2020). Systems-Level Immunomonitoring from Acute to Recovery Phase of Severe COVID-19. *Cell Rep. Med.* **1**, 100078.
 14. Wheatley, A.K., Juno, J.A., Wang, J.J., Selva, K.J., Reynaldi, A., Tan, H.X., Lee, W.S., Wrapp, K.M., Kelly, H.G., Esterbauer, R., et al. (2021). Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat. Commun.* **12**, 1162.
 15. Bergamaschi, L., Mescia, F., Turner, L., Hanson, A.L., Kotagiri, P., Dunmore, B.J., Ruffieux, H., De Sa, A., Huhn, O., Morgan, M.D., et al. (2021). Longitudinal analysis reveals that delayed bystander CD8+ T cell activation and early immune pathology distinguish severe COVID-19 from mild disease. *Immunity* **54**, 1257–1275.e8.
 16. Prebensen, C., Lefol, Y., Myhre, P.L., Lüders, T., Jonassen, C., Blomfeldt, A., Omrand, T., Nilsen, H., and Berdal, J.E. (2023). Author Correction: Longitudinal whole blood transcriptomic analysis characterizes neutrophil activation and interferon signaling in moderate and severe COVID-19. *Sci. Rep.*, **4**.
 17. Lindeboom, R.G.H., Worlock, K.B., Dratva, L.M., Yoshida, M., Scobie, D., Wagstaffe, H.R., Richardson, L., Wilbrey-Clark, A., Barnes, J.L., Kretschmer, L., et al. (2024). Human SARS-CoV-2 challenge uncovers local and systemic response dynamics. *Nature* **631**, 189–198.
 18. Duvignaud, A., Lhomme, E., Pistone, T., Onaisi, R., Sitta, R., Journot, V., Nguyen, D., Peiffer-Smadja, N., Crémer, A., Bouchet, S., et al. (2020). Home Treatment of Older People with Symptomatic SARS-CoV-2 Infection (COVID-19): A structured Summary of a Study Protocol for a Multi-Arm Multi-Stage (MAMS) Randomized Trial to Evaluate the Efficacy and Tolerability of Several Experimental Treatments to Reduce the Risk of Hospitalisation or Death in outpatients aged 65 years or older (COVERAGE trial). *Trials* **21**, 846.
 19. Duvignaud, A., Lhomme, E., Onaisi, R., Sitta, R., Gelly, A., Chastang, J., Piroth, L., Binquet, C., Dupouy, J., Makinson, A., et al. (2022). Inhaled ciclesonide for outpatient treatment of COVID-19 in adults at risk of adverse outcomes: a randomised controlled trial (COVERAGE). *Clin. Microbiol. Infect.* **28**, 1010–1016.
 20. Ahern, D.J., Ai, Z., Ainsworth, M., Allan, C., Allcock, A., and Angus, B. (2022). A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. *Cell* **185**, 916–938.
 21. Lin, X., Yao, Z., Sun, D., Lin, X., and Wang, X. (2023). Deconvolution algorithms for inference of the cell-type composition of the spatial transcriptome. *Comput. Struct. Biotechnol. J.* **21**, 176–184.
 22. Zhang, J.Y., Wang, X.M., Xing, X., Xu, Z., Zhang, C., Song, J.W., Fan, X., Xia, P., Fu, J.L., Wang, S.Y., et al. (2020). Single-cell landscape of immunological responses in patients with COVID-19. *Nat. Immunol.* **21**, 1107–1118.
 23. Arunachalam, P.S., Wimmers, F., Mok, C.K.P., Perera, R.A.P.M., Scott, M., Hagan, T., Sigal, N., Feng, Y., Bristow, L., Tak-Yin Tsang, O., et al. (2020). Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. *Science* **369**, 1210–1220.
 24. Tian, Y., Carpp, L.N., Miller, H.E.R., Zager, M., Newell, E.W., and Gottardo, R. (2022). Single-cell immunology of SARS-CoV-2 infection. *Nat. Biotechnol.* **40**, 30–41.
 25. Lucas, C., Wong, P., Klein, J., Castro, T.B.R., Silva, J., Sundaram, M., Ellingson, M.K., Mao, T., Oh, J.E., Israelow, B., et al. (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* **584**, 463–469.
 26. Mathew, D., Giles, J.R., Baxter, A.E., Oldridge, D.A., Greenplate, A.R., Wu, J.E., Alanio, C., Kuri-Cervantes, L., Pampena, M.B., D'Andrea, K., et al. (2020). Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science* **369**, eabc8511.
 27. Gauthier, M., Agniel, D., Thiébaud, R., and Hejblum, B.P. (2020). dearseq: a variance component score test for RNA-seq differential analysis that effectively controls the false discovery rate. *NAR Genom Bioinform* **2**, iqaa093.
 28. Wong, L.Y.R., and Perlman, S. (2022). Immune dysregulation and immunopathology induced by SARS-CoV-2 and related coronaviruses - are we our own worst enemy? *Nat. Rev. Immunol.* **22**, 47–56.
 29. Altman, M.C., Rinchai, D., Baldwin, N., Toufiq, M., Whalen, E., Garand, M., Syed Ahamed Kabeer, B., Alfaki, M., Presnell, S.R., Khaenam, P., et al. (2021). Development of a fixed module repertoire for the analysis and interpretation of blood transcriptome data. *Nat. Commun.* **12**, 4385.
 30. Hocini, H., Wiedemann, A., Blengio, F., Lefebvre, C., Cervantes-Gonzalez, M., Foucat, E., Tisserand, P., Surenada, M., Coléon, S., Prague, M., et al. (2023). Neutrophil Activation and Immune Thrombosis Profiles Persist in Convalescent COVID-19. *J. Clin. Immunol.* **43**, 882–893.
 31. Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.C., Uhl, S., Hoagland, D., Möller, R., Jordan, T.X., Oishi, K., Panis, M., Sachs, D., et al. (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* **181**, 1036–1045.e9.
 32. Sposito, B., Broggi, A., Pandolfi, L., Crotta, S., Clementi, N., Ferrarese, R., Sisti, S., Criscuolo, E., Spreafico, R., Long, J.M., et al. (2021). The interferon landscape along the respiratory tract impacts the severity of COVID-19. *Cell* **184**, 4953–4968.e16.
 33. Cobos, F.A., Panah, M.J.N., Epps, J., Long, X., Man, T.K., Chiu, H.S., Chomsky, E., Kiner, E., Krueger, M.J., di Bernardo, D., et al. (2023). Effective methods for bulk RNA-seq deconvolution using scRNA-seq transcriptomes. *Genome Biol.* **24**, 177.
 34. Wang, X., Park, J., Susztak, K., Zhang, N.R., and Li, M. (2019). Bulk tissue cell type deconvolution with multi-subject single-cell expression reference. *Nat. Commun.* **10**, 380.
 35. Newman, A.M., Liu, C.L., Green, M.R., Gentles, A.J., Feng, W., Xu, Y., Hoang, C.D., Diehn, M., and Alizadeh, A.A. (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nat. Methods* **12**, 453–457.
 36. Finotello, F., Mayer, C., Plattner, C., Laschober, G., Rieder, D., Hackl, H., Krogsdam, A., Loncova, Z., Posch, W., Wifflingseder, D., et al. (2019). Molecular and pharmacological modulators of the tumor immune contexture revealed by deconvolution of RNA-seq data. *Genome Med.* **11**, 34.
 37. Pascual, V., Chaussabel, D., and Banchereau, J. (2010). A Genomic Approach to Human Autoimmune Diseases. *Annu. Rev. Immunol.* **28**, 535–571.
 38. Berry, M.P.R., Graham, C.M., McNab, F.W., Xu, Z., Bloch, S.A.A., Oni, T., Wilkinson, K.A., Banchereau, R., Skinner, J., Wilkinson, R.J., et al. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* **466**, 973–977.
 39. Nakaya, H.I., Wrammert, J., Lee, E.K., Racioppi, L., Marie-Kunze, S., Haining, W.N., Means, A.R., Kasturi, S.P., Khan, N., Li, G.M., et al. (2011). Systems biology of vaccination for seasonal influenza in humans. *Nat. Immunol.* **12**, 786–795.
 40. Yang, Z.W., Yang, S.H., Chen, L., Qu, J., Zhu, J., and Tang, Z. (2001). Comparison of blood counts in venous, fingertip and arterial blood and their measurement variation. *Clin. Lab. Haematol.* **23**, 155–159.
 41. Eriksson, M., Sartono, E., Martins, C.L., Balé, C., Garly, M.L., Whittle, H., Aaby, P., Pedersen, B.K., Yazdanbakhsh, M., Erikstrup, C., and Benn, C.S. (2007). A comparison of ex vivo cytokine production in venous and capillary blood. *Clin. Exp. Immunol.* **150**, 469–476.

42. Berry, M.P.R., Graham, C.M., McNab, F.W., Xu, Z., Bloch, S.A.A., Oni, T., Wilkinson, K.A., Quinn, C., Blankenship, D., Dhawan, R., et al. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466, 973–977.
43. Nakaya, H.I., Wrammert, J., Lee, E.K., Racioppi, L., Marie-Kunze, S., Haining, W.N., Means, A.R., Kasturi, S.P., and Khan, N. (2011). Li G.M., et al. Systems biology of vaccination for seasonal influenza in humans. *Nat. Immunol.* 12, 786–795.
44. Ewels, P., Magnusson, M., Lundin, S., and Källér, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32, 3047–3048.
45. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120.
46. Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* 14, 417–419.

STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--|--|---|
| Biological samples | | |
| Human blood samples (venous and finger prick sampling) | French high-risk COVID-19 positive outpatients included in COVERAGE France trial | N/A |
| Chemicals, peptides, and recombinant proteins | | |
| Tempus™ RNA Stabilization Reagent | Applied Biosystems | Cat# 4342792 |
| GlobinClear™ Human Kit | Invitrogen (Ambion) | Cat# AM1980 |
| Critical commercial assays | | |
| finger-prick device | Sarstedt Safety Lancet Extra (18G, 1.8 mm) | Cat# 85.1018.200 |
| Bio-Plex Pro™ Human Cytokine Screening Panel (48-plex) | Bio-Rad | Cat# 12007283 |
| Human Premixed Multi-Analyte Magnetic Luminescence Assay (18-plex) | R&D Systems | Cat# LXSAHM-18 |
| Tempus™ Spin RNA Isolation Kit | Invitrogen | Cat# 4380204 |
| TruSeq® Stranded mRNA Library Prep Kit | Illumina | Cat# 20020595 |
| NEBNext® Single Cell/Low Input RNA Library Prep Kit | New England Biolabs | Cat# E6420 |
| LSRII Fortessa 4-laser flow cytometer | BD Biosciences | N/A |
| Bio-Plex 200 System | Bio-Rad | N/A |
| Illumina HiSeq 2500 V4 system | Illumina | N/A |
| Deposited data | | |
| Data of the study | This paper | BioStudies database - ebi.ac.uk (E-MTAB-15066) |
| Code of the analyzes | This paper | https://doi.org/10.5281/zenodo.14447221 |
| Software and algorithms | | |
| R (v4.3) and packages | CRAN/Bioconductor | Various |
| Bio-Plex Manager software version 6.0 | Bio Rad | https://www.bio-rad.com/fr-fr/product/bio-plex-manager-software-standard-edition |
| FlowJo software version 9.9.6 | Tree Star Inc. | https://flowjo.com/ |
| MultiQC v1.13 | Ewels et al. ⁴² | https://pypi.org/project/multiqc/1.13/ |
| Trimmomatic v17.0.11 | Bolger et al. ⁴³ | https://github.com/usadellab/Trimmomatic |
| Salmon v1.8.0 | Patro et al. ⁴⁴ | https://github.com/COMBINE-lab/salmon/releases |
| CIBERSORTx algorithm | Pascual et al. ³⁷ | https://cibersortx.stanford.edu/ |

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design and participants

The COVERAGE-Immuno study was embedded in the COVERAGE France trial, a phase-3, multicentre, open-label, randomized controlled trial of COVID-19 early treatment. The trial began in April 2020. The full original version of the protocol was previously published.^{18,19}

Criteria for inclusion in the trial are as follows: (a) age ≥ 60 years regardless of the presence of other risk factors, or ≥ 50 years with at least one of the following risk factors: high blood pressure, body mass index ≥ 30 kg/m², diabetes, ischemic heart disease, heart failure, history of stroke, chronic obstructive pulmonary disease, stage ≥ 3 chronic kidney disease, solid or haematological malignancy diagnosed < 5 years earlier, immunosuppressive therapy, or HIV infection with CD4 < 200 /mm³; (b) COVID-19 with first symptoms ≤ 7 days earlier; (c) positive SARS-CoV-2 nasopharyngeal RT-PCR or antigen test; (d) no criteria for hospitalization or acute oxygen therapy; and (e) written informed consent. Exclusion criteria are inability to understand or decide on participation; lack of

health insurance; and conditions or treatment contraindicating the use of one trial drugs. Participants who met all the inclusion criteria were randomly assigned at home or in an outpatient facility on day (D)0 to a control arm or one of the experimental arms ongoing at the time, and then visited on D1, D3, D5, D7, D9, D14, and D28.

COVERAGE-Immuno sub-study

Participants recruited at the sites were invited to take part in the COVERAGE-Immuno sub-study, including additional sampling for in-depth, repeated evaluation of the immunological markers and gene expression data (Figure S1). Blood samples through veno-puncture were realized by a nurse at D0, D7 and D14 for the quantification of cell phenotype by flow cytometry on fresh blood (1 EDTA tube), serum analytes (1 dry tube) and gene expression (1 tempus tube). At D0, each patient was educated to self-sample by using the finger-prick at home (Figure S1). Hence, self-collected finger-prick capillary blood samples were harvested at D0, D1, D3, D5, D7, D9, D12, and D14. Cotton swabs were stored in freezers and then collected weekly by a nurse.

Sex at birth was recorded for all participants at inclusion. Due to the limited sample size, the study was not powered to formally assess sex- or gender-specific differences in immune or transcriptomic responses. Therefore, analyses were not stratified by sex, and potential sex- or gender-related effects should be interpreted with caution.

METHOD DETAILS

Ethics

The trial protocol was approved by a French Ethics Committee (CPPIDF1-2020-ND45). Written informed consent was obtained from all participants.

Role of funders

The COVERAGE-Immuno study was funded by EIT Health (Grant number: 20874 COVERAGE-Immuno). The funder had no role in the study design, data collection, analysis, interpretation, manuscript writing, or decision to submit for publication.

Venous sampling and processing

For the biological samples, blood was collected using standardized sampling kits (EDTA tube, Dry tube, Tempus Tube or self-tests). Blood samples are then transported at controlled temperature (+25°C) except for the blood self-test (+5°C) to the Biological Ressource Center (BRC) of Bordeaux Hospital. The EDTA blood tube are directly transported to the immunomonitoring platform for cytometric analysis. After quality control, the BRC process samples under sterile conditions, according to COVERAGE sample management procedure to obtain Serum from dry blood tube after centrifugation 10 minutes at 2500g at room temperature. Samples are aliquoted in 300uL into 1D barcoded, biocompatible and suitable to cryostorage cryotubes. Tempus, blood self-test Tubes or serum aliquots are stored at -80°C within 6 hours following the sampling to guarantee their integrity and long-term storage. Data related to bio-sampling processes and non-conformity are recorded in MBioLIMS® database to ensure traceability. The BRC are certified according to biobank standards ISO 20387.

Finger-prick sampling

Blood samples were self-collected by patients using a finger-prick device (Sarstedt Safety lancet, Extra, Ø needle: 18 G, penetration depth: 1.8 mm). The blood was collected onto a cotton swab, which was immediately placed into a tube containing 200 µL of Tempus RNA stabilization reagent (Applied Biosystems). The collection tubes were stored in the patient's home freezer before being transported to the clinical investigation centre in insulated packaging. RNA extractions and all subsequent sample processing steps, for both Tempus and cotton swab samples, were performed as single analyses.

Quantification of serum analytes

Serum analytes were measured at baseline, 7 days and 14 days with samples collected during visits at home.

Sixty-six Th1/Th2/activation/inflammation/apoptosis markers were measured in sera by Luminex assay, allowing the detection of 54 analytes with commercial kits (48-plex Bio-Plex Pro Human Cytokine screening Panel, Bio-Rad and 18-plex Human premixed Multi-Analyte magnetic Luminex Assay, R&D) according to the manufacturer's instructions kits. Median fluorescence intensity for each sample was measured using the Bio-Plex 200 system (Bio-Rad, Marnes-la-Coquette, France). The Bio-Plex Manager software version 6.0, incorporating a weighted five-parameter logistic curve-fitting method, was used to calculate sample concentrations (in pg/ml). Twelve biomarkers showing more than 20% of undetectable data (below lower limit of quantification) were removed from the analysis. A total of 54 analytes was analysed.

Cell phenotyping

All immunogenicity assays were performed on frozen peripheral blood mononuclear cells (PBMC). Immune phenotyping of the different cell populations was performed with an LSRII Fortessa 4-laser (488, 640, 561 and 405 nm) flow cytometer (BD Biosciences), and FlowJo software version 9.9.6 (Tree Star Inc.). i) CD4⁺ and CD8⁺T cells were analyzed for CD45RA and CC-chemokine receptor 7 (CCR7) expression, to identify the naive (CD45RA+CCR7+), central memory (CD45RA-CCR7+), effector memory (CD45RA-CCR7-)

and terminal effector (CD45RA+CCR7-) cell subsets, and for co-expression of the activation markers HLA-DR and CD38 ; ii) B-cell subsets were analyzed with the CD19, CD21, CD27, CD38, immunoglobulin (Ig) D and IgM markers. Antibody-secreting cells are identified as CD19⁺ cells expressing CD38 and CD27; iii) NK cell subsets were analyzed using CD16, CD56 and CD57 markers, as well as Ki 67 to define proliferating NK cells; iv) HLA-DR, CD33, CD45RA, CD123, CD141 and CD1c were used to identify DC subsets, myeloid DC, plasmacytoid DC, DC1 and DC2.

RNA sequencing

Gene expression profile was measured in Tempus samples at baseline, D7 and D14 as well as in cotton swab at the same time points, plus D3, D5, D9, D12.

Samples were analysed for gene expression profiles by mRNA sequencing. Total RNA was purified from whole blood using TempusTM Spin RNA Isolation Kit (Invitrogen) and Globin mRNAs removed by GLOBINclear-Human Kit (Ambion). RNAs were then quantified with Quant-iT RiboGreen RNA Assay Kit (Thermo Fisher Scientific) before to be controlled for quality on the Bioanalyzer (Agilent). mRNA libraries were prepared by the TruSeq[®] Stranded mRNA kit according to Illumina protocol for Tempus whole blood recovery, and by the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina for cotton prick samples. Libraries were sequenced on Illumina HiSeq 2500 V4 system. The sequencing depth were about 50 million reads and the fragment length of 1X100bp.

Quality control of the alignment was performed via MultiQC v1.13.⁴² After this control stage, Trimmomatic v1.0.11⁴³ was used to trim reads from sequences below a threshold, trim adapters and trim reads smaller than a defined length. The reads were aligned to the v44 human reference transcriptome (GRCh38.p14 from GenCode) using Salmon v1.8.0⁴⁴ and quantified relative to annotation model “hsapiens_gene_ensembl” recovered from the R package biomaRt v2.58.0.⁴⁵ Finally, counts were normalized as counts per million.

QUANTIFICATION AND STATISTICAL ANALYSIS

The concentration of 54 measured cytokines and the cellular responses assessed at D0, D7 and D14 were compared between baseline (D0) and the two other times separately using the Wilcoxon signed-rank test. We used the FDR method (Benjamini-Hochberg method) to adjust for test multiplicity.

For the transcriptomic part, differential expression analysis was carried out using the dearseq R package²⁷ to analyze gene expression levels that differed between baseline (D0) and, respectively, D7 and D14 for finger-prick and tempus samples, separately. After the gene-level analysis, we focused on the gene set level with predefined gene sets: a fixed repertoire of 382 transcriptional modules that were thoroughly functionally annotated, “BloodGen3”.²⁸ From this, we conducted an analysis using the differential gene set function of the dearseq R package⁴⁶ and visualized the results on fingerprint grids using the fold change (FC) ($|FC| > 1.5$). Briefly, dearseq uses a one step statistical procedure that directly test the significance of a whole gene set by a gene-set variance component score test. The criteria for calling a gene set as differentially expressed is its Benjamini-Hochberg adjusted p-value being under 1%. For finger-prick samples only, gene expression assessed at D0, D1, D3, D5, D7, D9, D12, D14 was analyzed for the gene and gene set levels with the same method previously described.

Cell deconvolution was inferred using CIBERSORTx algorithm³⁷ and the TIL10³⁸ matrix reference. The estimated proportions were compared with the cell phenotyping using root-mean-square-error (RMSE) and Pearson correlation. Agreement between estimated and observed proportions of cells or between results from finger-prick and venous sampling was measured by intraclass correlation coefficient (CCI) and concordance correlation coefficient (CCC). These comparisons are shown in scatter plots and Blant-Altman plots where limits of agreement were quantified.

Analyses were performed using R software (the R Foundation, Vienna, Austria; version 4.3).

ADDITIONAL RESOURCES

The trial protocol was registered in the European database EudraCT (2020-001435-27) and on clinicaltrials.gov (NCT04356495).