A serum calprotectin lateral flow test as an inflammatory and prognostic marker in acute lung infection: a prospective observational study

Received: 17 Jan 2024
Accepted: 5 Feb 2024

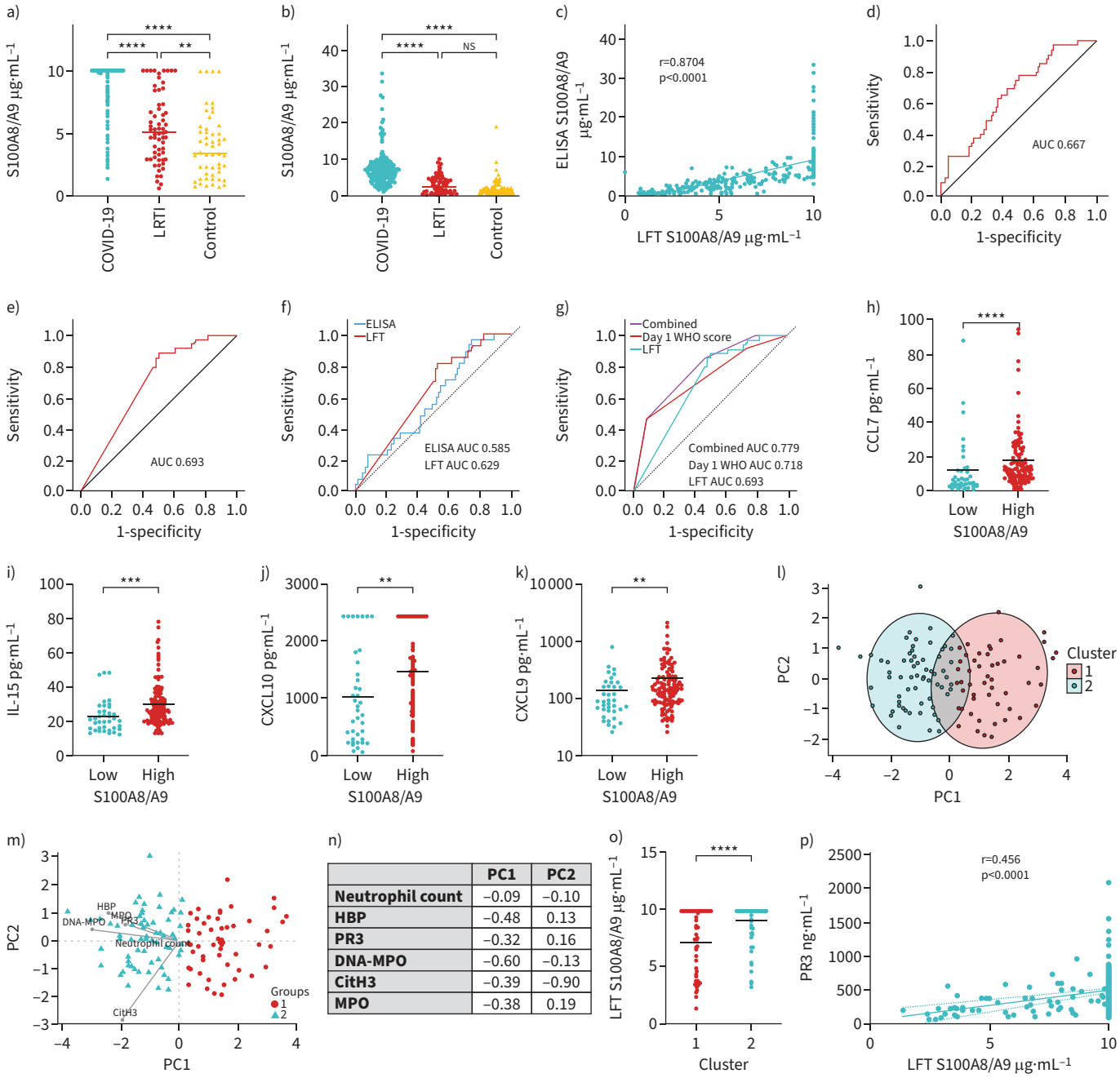
To the Editor:

Excess neutrophilic inflammation is associated with poor prognosis in a number of acute and chronic respiratory infections [1]. Increased numbers of circulating neutrophils are observed during acute respiratory tract infections including COVID-19, where in particular increased numbers of immature neutrophils are associated with more severe disease [2]. S100A8/A9 (calprotectin) is an antimicrobial complex found in the cytoplasm and granule membranes of neutrophils, and is released by activated neutrophils during neutrophil extracellular trap (NET)osis [3]. Serum S100A8/A9 levels are increased in sepsis [4] and COVID-19 [5–7], as well as other acute [8] and chronic lung diseases [9, 10], and correlate with disease severity [11]. COVID-19 presentation ranges from asymptomatic infection to severe disease requiring invasive mechanical ventilation, and previous research suggests that the extent of neutrophil activation can predict poor clinical outcomes [12]. A rapid biomarker test for neutrophil activation that is convenient to perform and able to predict outcomes in multiple diseases, including COVID-19, would be useful for clinical decision making. Such a test may be used to direct the use of neutrophil-targeting treatments in the future. In this study, we investigated the clinical utility of a lateral flow test (LFT) for S100A8/A9 in people with COVID-19, non-COVID-19 lower respiratory tract infection (LRTI) and matched controls.

We conducted a prospective observational study of adults admitted to hospital with acute respiratory infection. All patients were recruited within 96 h of hospital admission from two centres, Ninewells Hospital (Dundee, UK) and Sheffield Teaching Hospitals (Sheffield, UK). All patients underwent combined nasal and pharyngeal swab for COVID-19 PCR testing. “Healthy” controls were recruited from hospital inpatients without infection predominantly awaiting elective procedures such as cardiac or orthopaedic surgery. Clinical status and severity were based on the World Health Organization (WHO) seven-point ordinal scale (1: not hospitalised and no limitations on activities; 2: not hospitalised and limitations on activities; 3: hospitalised and not requiring supplemental oxygen; 4: hospitalised and requiring supplemental oxygen; 5: hospitalised and on noninvasive ventilation or high-flow oxygen devices; 6: hospitalised and on invasive mechanical ventilation or extracorporeal membrane oxygenation; 7: death). Patients were eligible for enrolment if they had a clinical severity of 3–5 on the WHO scale at baseline. Outcomes were evaluated at day 29 after enrolment. Serum samples at baseline were used to measure S100A8/A9 levels quantitively using the Quantum Blue sCAL kit (Bühlmann) LFT and Human S100A8/S100A9 Heterodimer DuoSet ELISA (R&D Systems). 45 serum cytokines were measured using the Olink Target 48 cytokine panel. Additional neutrophil markers, including DNA–myeloperoxidase (MPO) complexes [13], heparin-binding protein (HBP/AZU1) (Axis-Shield), MPO (R&D Systems), citrullinated histone H3 (CitH3; Cayman Chemical) and proteinase 3 (PR3) (R&D systems), were measured by ELISA. Comparisons across the three study groups utilised the Kruskal–Wallis test while proportions were compared by Chi-squared test. Discrimination for clinical outcomes was determined using the area under the receiver operator characteristic (ROC) curve (AUC). A p-value <0.05 was considered statistically significant for all analyses. For comparison of cytokine levels between two groups, Mann–Whitney test and false discovery rate (FDR) <0.05 were utilised. Ethical approval was obtained from the East of Scotland Research Ethics Committee (REC number 20/ES/0055).

Shareable abstract (@ERSpublications)
A rapid, quantitative serum S100A8/A9 (calprotectin) lateral flow test in combination with clinical status predicted outcomes in people hospitalised with COVID-19 and associated with a patient cluster driven by markers of neutrophil activation https://bit.ly/48e1Blv

Cite this article as: Gilmour A, Hughes C, Giam YH, *et al*. A serum calprotectin lateral flow test as an inflammatory and prognostic marker in acute lung infection: a prospective observational study. *ERJ Open Res* 2024; 10: 00059-2024 [DOI: 10.1183/23120541.00059-2024].



n)

	PC1	PC2
Neutrophil count	−0.09	−0.10
HBP	−0.48	0.13
PR3	−0.32	0.16
DNA-MPO	−0.60	−0.13
CitH3	−0.39	−0.90
MPO	−0.38	0.19

FIGURE 1 Serum S100A8/A9 (calprotectin) measured by quantitative lateral flow test (LFT) and ELISA in acute lung infection, and associations with inflammation and clinical outcomes. S100A8/A9 levels in COVID-19 (n=157), matched controls (n=52) and non-COVID lower respiratory tract infection (LRTI) (n=67), measured by a) LFT or b) ELISA. c) Correlation between S100A8/A9 concentration from LFT and ELISA (n=274). Receiver operating characteristic (ROC) curves for prediction of severe COVID-19 defined as World Health Organization (WHO) score 4–7 (*i.e.* requiring supplementary oxygen or ventilation, or death by day 29) (WHO score 1–3, n=133; WHO score 4–7, n=37) with S100A8/A9 d) ELISA and e) LFT. f) ROC curve indicating predictive value of S100A8/A9 ELISA and LFT for COVID-19 patient 29-day mortality. g) ROC curve for prediction of severe COVID-19 at day 29 using combination of day 1 WHO score and 10 µg·mL^{-1} S100A8/A9 LFT cut-off. Levels of inflammatory cytokines h) C-C motif chemokine ligand (CCL)7, i) interleukin (IL)-15, j) CXC motif chemokine ligand (CXCL)10 and k) CXCL9 in COVID-19 patients stratified by low (n=38, quartile 1) and high (n=112, quartiles 2–4) levels of serum S100A8/A9. l) Principal component (PC) analysis and m) biplot indicating two clusters based on serum neutrophil associated markers proteinase 3 (PR3), heparin-binding protein (HBP), citrullinated histone 3 (CitH3), DNA–myeloperoxidase (MPO) and peripheral blood neutrophil count (cluster 1, n=49; cluster 2, n=65); K-means clustering. n) PC1 and PC2 scores for each neutrophil marker. o) S100A8/A9 LFT levels of individuals in cluster 1 and 2. p) Correlation between serum LFT S100A8/A9 levels and neutrophil protease PR3 in COVID-19. NS: nonsignificant; AUC: area under the ROC curve. **: p<0.01; ***: p<0.001; ****: p<0.0001 by Mann–Whitney test.

225 hospitalised patients were included; 158 patients (mean±SD age 64.5±13 years, 60.5% male) with PCR-confirmed COVID-19 patients and 67 hospitalised patients with non-SARS-CoV-2 LRTI (69.4±13.3 years, 49.2% male). The control group consisted of 52 non-infection matched controls (62.5±14.3 years, 48.1% male). LRTI patients were significantly older than COVID-19 patients (p=0.01) and the control group (p=0.0126). There was no significant difference in gender between these groups (p=0.145). In the COVID-19 and LRTI groups, day 1 WHO scores were: 34 (21.5%) and 32 (47.8%) with WHO 3; 97 (61.4%) and 31 (46.3%) with WHO 4; and 26 (16.5%) and three (4.5%) with WHO 5, respectively.

S100A8/A9 levels in serum were significantly increased in COVID-19 (LFT/ELISA: 8.3±2.4/7.7±5.6 μg·mL^{-1}) *versus* LRTI patients (4.67±2.74/1.98±2.65 μg·mL^{-1}) and controls (4.0±2.5/2.5±2.8 μg·mL^{-1}) ($p \leqslant 0.0001$) (figure 1a and b). S100A8/A9 levels measured by ELISA and LFT assays were strongly correlated (r=0.87, $p \leqslant 0.0001$) (figure 1c). In COVID-19 patients, ROC analysis showed that both S100A8/A9 ELISA and LFT values were moderately predictive of lack of recovery at day 29 (defined as ongoing requirement for supplemental oxygen or death at day 29 (WHO groups 4–7)) (ELISA AUC 0.66, 95% CI 0.57–0.77; LFT AUC 0.69, 95% CI 0.60–0.78) (figure 1d and e). LFT S100A8/A9 levels were also moderately predictive of 29-day mortality (n=25) in COVID-19, which was not demonstrated for the ELISA (ELISA AUC 0.59, 95% CI 0.47–0.70; LFT AUC 0.63, 95% CI 0.53–0.73) (figure 1f). Combining day 1 WHO score with LFT S100A8/A9 level of above or below 10 μg·mL^{-1} (the optimal cut-off identified using Youden's index) increased the predictive power for severe disease or mortality by day 29 (combined AUC 0.779 (95% CI 0.694–0.864; compared to day 1 WHO alone AUC 0.718, 95% CI 0.598–0.777; or LFT alone AUC 0.693, 95% CI 0.515–0.822) (figure 1g). Neither LFT or ELISA S100A8/A9 levels had significant predictive value for mortality or severity in LRTI (data not shown).

Intravascular neutrophil activation and inflammation have been reported to be higher in COVID-19 compared to other respiratory infections, including influenza pneumonia, which may explain the increased levels of serum S100A8/A9 in these patients [14]. Therefore, we focused on the COVID-19 group to further understand the associations between S100A8/A9 and inflammation. We divided patients into high (above quartile 1) and low (below quartile 1) S100A8/A9 levels based on their distribution in COVID-19 patients. Key cytokines with significant differences between these groups included the C-C motif chemokine ligand 7 (p<0.0001) (figure 1h), interleukin (IL)-15 (p=0.0003) (figure 1i), CXC-motif chemokine ligand (CXCL)10 (IP-10) (p=0.0026) (figure 1j), CXCL9 (p=0.003) (figure 1k) and IL-6 (p=0.006), and 13 cytokines were significantly different (FDR<0.05) in total (data not shown).

Based on blood neutrophil count and neutrophil-associated serum markers, two clusters were identified (cluster 1 and 2) within the COVID-19 group (figure 1l). Principal component analysis demonstrated that cluster 2 was driven by high levels of neutrophil markers including NET markers, DNA–MPO complexes and CitH3, as well as the neutrophil proteins MPO, HBP and PR3 (figure 1m and n). Cluster 2 had significantly higher S100A8/A9 levels than cluster 1 (p<0.0001) (figure 1o). S100A8/A9 showed significant, moderate correlation with multiple other neutrophil markers (figure 1p).

Our results therefore show that a rapid LFT for S100A8/A9, which can provide a result within 1 h, is moderately associated with severity of COVID-19, and indicates a hyperinflammatory subset with multiple increased neutrophil markers and proinflammatory cytokines at baseline. Our results support other findings that reported serum S100A8/A9 levels predicted the need for invasive mechanical ventilation and intensive care unit admission in hospitalised COVID-19 patients [15], and can be used in combination with day 1 WHO score to enhance the predictive power of this routinely used clinical score. Our study is limited by the LFT upper limit of quantification being 10 μg·mL^{-1}, which could be improved by use of an extended-range test or by further sample dilution. Our study was performed during the first and second waves of COVID-19 in the UK, and so does not include data from patients with the current circulating Omicron variant or following vaccination. Nevertheless, this study suggests the potential for use of a point-of-care prognostic biomarker, which may be relevant to investigate further in other severe, acute, neutrophil-associated diseases. The potential application of such a biomarker to clinical decision making requires further study.

Amy Gilmour[1,3], Chloe Hughes[1,3], Yan Hui Giam[1], Rebecca C. Hull[1,2], Thomas Pembridge[1], Hani Abo-Leyah[1], A.A. Roger Thompson[2], Alison M. Condliffe[2], Amelia Shoemark[1], James D. Chalmers[1] and Merete B. Long[1]

[1]Molecular and Clinical Medicine, University of Dundee, Dundee, UK. [2]University of Sheffield, Sheffield, UK. [3]These authors contributed equally.

Corresponding author: Merete B. Long (MLong001@dundee.ac.uk)

Provenance: Submitted article, peer reviewed.

Ethics statement: Ethical approval was obtained from the East of Scotland Research Ethics Committee (REC number 20/ES/0055).

Conflict of interest: A.A.R. Thompson reports grants or contracts from British Heart Foundation, Heart Research UK, and National Institute for Health and Care Research, outside the submitted work; honoraria for lectures from Janssen-Cilag Ltd, outside the submitted work; and support received for travel and conference registration from Janssen-Cilag Ltd, outside the submitted work. A.M. Condliffe reports grants or contracts from NIHR and MRC, outside the submitted work. A. Shoemark reports grants or contracts from AstraZeneca, outside the submitted work; consulting fees from Spirovant and Translate Bio, outside the submitted work; payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events for Translate Bio, Ethris and Insmed, outside the submitted work; and involvement in European Respiratory Society Clinical Research Collaborations (EMBARC, BEATPCD and AMR), EMBARC3 is supported by project partners Armata, AstraZeneca, Boehringer Ingelheim, Chiesi, CSL Behring, Grifols, Insmed, Janssen, Lifearc, Novartis, and Zambon, disclosures made outside the submitted work. J.D. Chalmers reports grants or contracts from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Gilead Sciences, Grifols, Novartis, Insmed and Trudell, outside the submitted work; and consulting fees from Antabio, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Insmed, Janssen, Novartis, Pfizer, Trudell and Zambon, outside the submitted work; and is an associate editor of this journal. The remaining authors have nothing to disclose.

Support statement: This study was funded by the University of Dundee Coronavirus Research Fund. J.D. Chalmers is supported by the Asthma and Lung UK Chair of Respiratory Research. Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Keir HR, Shoemark A, Dicker AJ, *et al.* Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. *Lancet Respir Med* 2021; 9: 873–884.
2 Aschenbrenner AC, Mouktaroudi M, Krämer B, *et al.* Disease severity-specific neutrophil signatures in blood transcriptomes stratify COVID-19 patients. *Genome Med* 2021; 13: 7.
3 Sprenkeler EGG, Zandstra J, van Kleef ND, *et al.* S100A8/A9 Is a marker for the release of neutrophil extracellular traps and induces neutrophil activation. *Cells* 2022; 11: 236.
4 Gao RY, Jia HM, Han YZ, *et al.* Calprotectin as a diagnostic marker for sepsis: a meta-analysis. *Front Cell Infect Microbiol* 2022; 12: 1045636.
5 Norman GL, Navaz SA, Kanthi Y, *et al.* Circulating calprotectin as a predictive and severity biomarker in patients with COVID-19. *Diagnostics (Basel)* 2022; 12: 1324.
6 Mao Q, Wang C, Wen W, *et al.* A meta-analysis of the association between calprotectin and the severity of COVID-19. *J Infect* 2022; 84: e31–e33.
7 Renieris G, Karakike E, Gkavogianni T, *et al.* IL-1 mediates tissue-specific inflammation and severe respiratory failure in COVID-19. *J Innate Immun* 2022; 14: 643–656.
8 Havelka A, Sejersen K, Venge P, *et al.* Calprotectin, a new biomarker for diagnosis of acute respiratory infections. *Sci Rep* 2020; 10: 4208.
9 Titova EV, Løfblad L, Åsberg A, *et al.* Calprotectin as a biomarker of pneumonia in patients hospitalized with AECOPD. *Eur Respir J* 2019; 54: Suppl. 63, PA2895.
10 Machahua C, Guler SA, Horn MP, *et al.* Serum calprotectin as new biomarker for disease severity in idiopathic pulmonary fibrosis: a cross-sectional study in two independent cohorts. *BMJ Open Respir Res* 2021; 8: e000827.
11 Cardiero G, Palma D, Vano M, *et al.* Calprotectin levels and neutrophil count are prognostic markers of mortality in COVID-19 patients. *Diagnostics (Basel)* 2022; 12: 2554.
12 Meizlish ML, Pine AB, Bishai JD, *et al.* A neutrophil activation signature predicts critical illness and mortality in COVID-19. *Blood Adv* 2021; 5: 1164–1177.
13 Maruchi Y, Tsuda M, Mori H, *et al.* Plasma myeloperoxidase-conjugated DNA level predicts outcomes and organ dysfunction in patients with septic shock. *Crit Care* 2018; 22: 176.
14 Nicolai L, Leunig A, Brambs S, *et al.* Vascular neutrophilic inflammation and immunothrombosis distinguish severe COVID-19 from influenza pneumonia. *J Thromb Haemost* 2021; 19: 574–581.
15 García de Guadiana-Romualdo L, Rodríguez Rojas C, Morell-García D, *et al.* Circulating levels of calprotectin, a signature of neutrophil activation in prediction of severe respiratory failure in COVID-19 patients: a multicenter, prospective study (CalCov study). *Inflamm Res* 2022; 71: 57–67.