Received: 9 June 2022 | Revised: 29 September 2022 | Accepted: 3 October 2022

DOI: 10.1111/ejh.13881

CASE REPORT

European Journal of Haematology

WILEY

Phenotype of BTK-lacking myeloid cells during prolonged COVID-19 and upon convalescent plasma

André M. C. Gomes[1] | Guilherme B. Farias[1] | Amelia C. Trombetta[1] | Ana Godinho-Santos[1] | Inês Parreira[2] | Hélder Diogo Gonçalves[2] | Mariana Lessa Simões[3] | Patrício Aguiar[3] | Maria Manuel Deveza[4] | João Inácio[5] | Ana E. Sousa[1,6] | Susana Lopes da Silva[1,6,7]

[1]Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

[2]Serviço de Medicina II, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal

[3]Serviço de Medicina I, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal

[4]Serviço de Imuno-Hemoterapia, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal

[5]Serviço de Imagiologia Geral, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal

[6]Centro de Imunodeficiências Primárias, Centro Académico de Medicina de Lisboa, Lisbon, Portugal

[7]Serviço de Imunoalergologia, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal

Correspondence
Susana Lopes da Silva, Universidade de Lisboa, 1649-004 Lisbon, Portugal, Centro Académico de Medicina de Lisboa, 1649-028 Lisbon, Portugal, and Centro Hospitalar Universitário Lisboa Norte, 1649-028 Lisbon, Portugal.
Email: susanasilva@medicina.ulisboa.pt

Funding information
Fundação para a Ciência e a Tecnologia; Janssen Pharmaceuticals

Abstract

XLA patient with 7-month course of COVID-19 with persistent plasma SARS-CoV-2 load revealed a sustained non-inflammatory profile of myeloid cells in association with contained severity of disease, arguing in favor of the use of BTK inhibitors in SARS-COV-2 infection.

KEYWORDS
BTK, COVID-19, innate immunity, monocytes, SARS-CoV-2, X-linked agammaglobulinemia

Patients with X-linked agammaglobulinemia (XLA) may feature a severe course of SARS-CoV-2 infection that has been mainly attributed to the lack of B cells, supporting the use of plasma from convalescent individuals to replace the missing humoral response.[1]

XLA is caused by mutations in the Bruton tyrosine kinase (BTK), which in addition to its essential role in B-cell development, impacts in many pathways in monocytes, namely TLR signaling, cytokine production, and modulation of M1-like pro-inflammatory profile and M2-like immuno-regulatory phenotype.[2]

Monocyte/macrophages are key determinants of the evolution of SARS-CoV-2 infection, contributing to the lung disruption associated with pneumonia, as well as to the systemic pro-inflammatory state and the cytokine storm associated with worst prognosis.[3] On the other hand, we found an M2-like shift of circulating monocytes during the recovery of severe COVID-19 that may be linked to tissue repair.[3]

Notably, BTK has been emerging as a possible therapeutic target, based on the positive association found between BTK activity and severity of SARS-CoV-2 infection, and the observed decrease in inflammatory markers and improved clinical outcomes in COVID-19 patients under treatment with BTK inhibitors in the context of other concomitant diseases.[4]

These findings prompted us to investigate the myeloid phenotype in a 35-year-old XLA patient that featured an extremely prolonged disease course with persistent SARS-CoV-2 viral load for more than 7 months (Table 1), documented both in nasopharyngeal swabs,

TABLE 1 Clinical and epidemiological data from patients and healthy controls

	Patient with X-linked agammaglobulinemia (study timepoints)					
	1	2	3	4	COVID-19 Cohort[a]	Healthy controls
Number (male)	1 (1)				20 (17)	11 (9)
Age (years)	35				55.5 (39–65)	58 (39–65)
Time from symptom start (days)	75	145	212	217[b]	8.5 (5–11)	NA
CRP (mg/dl)	0.7	8.9	6.1	1.6	8.8 (4.85–25.5)	ND
PCT (ng/ml)	0.03	0.03	0.05	<0.02	0.16 (0.11–0.38)	ND
Ferritin (ng/ml)	1827	1600	1430	1539	939 (402–1906)	ND
Interleukin-6 (pg/ml)	6.3	ND	33.5	1.8	18 (4.5–36)	0.85 (0.24–1.6)
Total serum IgG (mg/dl)	744	655	735	1518		
Lymphocytes/μl	2556	1113	1697	2165	920 (845–1662)	1940 (1423–2200)
$CD4^+$ T cells/μl	1140	402	304	854	247 (133–392)	768 (544–998)
$CD8^+$ T cells/μl	1007	306	853	894	145 (81.2–262)	414 (158–577)
CD4/CD8 ratio	1.13	1.11	0.36	0.95	1.78 (0.93–2.50)	1.95 (1.53–4.17)
Neutrophils/μl	2916	7186	14 515	3339	4251 (2413–6917)	3228 (2521–6390)
Lymphocytes/neutrophils ratio	0.88	0.15	0.12	0.65	0.23 (0.15–0.50)	0.51 (0.47–0.61)
Monocytes/μl	480	356	487	348	349 (223–537)	398 (275–733)
Basophils/μl	12	10	17	12	20 (10–35)	32 (16–63)
Eosinophils/μl	36	17	50	35	13 (7–56)	115 (96–297)
SARS-CoV-2 plasma viral load (RNA cps/ml)	23	201	116	ULoD	112 (24–498)[c]	NA

Note: Values expressed as medians (interquartile range) unless otherwise specified.
Abbreviations: CRP, C reactive protein; NA, not applicable; ND, not done; PCT, procalcitonin; ULoD, under limit of detection.
[a]COVID-19 associated co-morbidities: arterial hypertension 9 (45%); Diabetes type II 6 (30%); Obesity 6 (30%); Lung emphysema 2 (10%).
[b]Convalescent plasma was administered at Days 213 and 214 after starting of symptoms.
[c]Quantified in the 13 (65%) COVID-19 patients with detectable plasma viremia.

assessed by RT-PCR, and in the plasma, quantified by ddPCR.[3] Data were compared with 20 COVID-19 patients evaluated at hospital admission and 13 healthy subjects recruited in parallel, that have been included in recently published studies,[3,5] and whose clinical and epidemiological data are summarized in Table 1. As expected, the hospitalized Covid-19 patients were older than the XLA patient, but were used as illustrative of the immunological alterations associated with severe COVID-19. All samples were processed immediately after collection, and the staining performed in whole blood allowed the analysis of a large number of cells by flow cytometry using both unsupervised and manual approaches, as previously described.[3,5]

The XLA patient features a c.1559G>A mutation in the exon 15 of the *BTK* gene. Intravenous IgG (IVIG) replacement therapy was started at the age of 4 after septic hip and recurrent respiratory infections, maintaining IgG serum level above 800 mg/dl, without major infections besides occasional sinusitis. At the age of 31, he was diagnosed with type 1 diabetes, with difficult metabolic control due to poor compliance with diet (overweigh since adolescence, BMI 29 kg/m^2) and treatment (glycosylated hemoglobin A1c around 11%).

SARS-CoV-2 infection manifestations were fever with cough and dyspnea. Chest CT scan performed on the 8th day of disease revealed multifocal lung ground glass opacities that slowly progressed over more than 7 months leading to involvement of up to 70% of both lungs, with a radiological pattern of organizing pneumonia (Figure 1A), despite steroid therapy started after day 35 of disease (mean 0.4 mg/kg). The imaging alterations could not be ascribed to other infections, given the lack of microbial isolation and the absence of response to broad-spectrum antibiotics. Peripheral O_2 saturation was never below 85% and therefore there was only need for low-flow nasal cannula oxygen and no need for any form of non-invasive/invasive ventilation or ICU admission.

It is therefore likely that the BTK defect contributed to this smoldering relatively protected course despite the presence of co-morbidities associated with an adverse prognosis. Although the loss of function of BTK may impact the biology of many cell populations, monocytes are probable main culprits given their role in COVID-19.[2,3] We evaluated longitudinally the myeloid compartment by flow cytometry, as illustrated in Figure S1A, and found lower expression of several activation markers like HLA-DR, PD-L1, CD86, and CD80 as compared with patients with moderate to severe COVID-19 and healthy individuals, in both the unsupervised approach to total monocytes (Figure 1B), and the manual analysis of monocyte subsets (Figure 1C). Additionally, there was marked reduction in Slan+ non-classical monocytes (Figure 1C), a sub-population involved in inflammatory conditions but shown to be reduced in COVID-19.[3,5] The levels of HLA-DR were also reduced in plasmacytoid dendritic cells

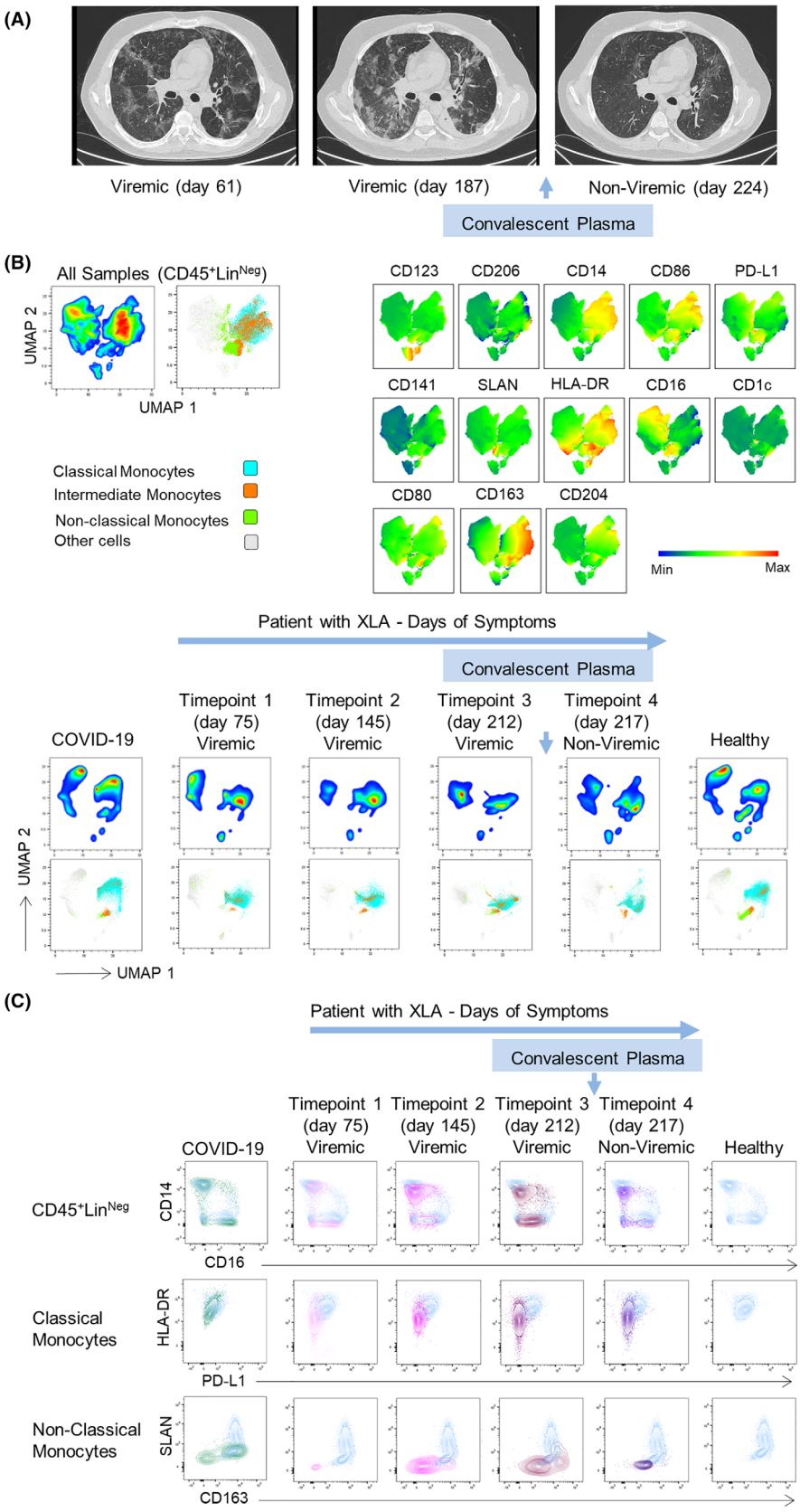

FIGURE 1 Longitudinal lung imaging and monocyte profile in SARS-CoV-2-infected XLA patient treated with convalescent plasma. (A) Chest CT. (B) Unsupervised analysis, UMAP of $CD45^{+}Lineage^{Neg}$ cells, marker expression, and manual annotation; relative subset distribution in patient timepoints and representative COVID-19 and healthy individuals. (C) Monocyte manual analysis showing CD16/CD14 within $CD45^{+}Lineage^{Neg}$ cells (top); HLA-DR/PD-L1 in classical (middle); and Slan/CD163 in non-classical (bottom) monocytes.

(DCs), as well as in CD141+ and CD1c+ DCs (Figure S1B). These findings contrasted with the progressive increase observed in serum IL-6 levels and CRP (Table 1), as well as the increase in neutrophils and decline in lymphocyte counts, which have been associated with adverse prognosis of COVID-19.[3,5] Regarding T cells, the flow cytometry analysis revealed the expected expansion of memory-effector CD4 and CD8 T-cell subsets (Figure S2). Therefore, our data support a contribution of the reduced myeloid activation to the slow disease

progression throughout the 7-month course of the SARS-CoV-2 infection.

Remdesivir (Day 64, 5-day course) had no impact. A clear reduction in inflammatory markers was only observed upon treatment with convalescent plasma (Days 213 and 214, Table 1). The plasma viral load was undetectable after 3 days (Table 1), although SARS-Cov-2 was found in a control bronchoalveolar lavage performed at Day 221 (643 382 RNA cps/ml by ddPCR) and the nasopharyngeal swabs remained positive for 60 days more. The impact of convalescent plasma on the myeloid profile was more evident on the recovery of intermediate and non-classical monocyte populations and DCs (Figure 1). There was a progressive clinical/laboratorial improvement, allowing steroid tapering (stopped on Day 292) and gradual resolution of chest CT lung opacities (Figure 1A). Functional recovery also supports a potential contribution of the BTK defect for the prevention of lung fibrosis.

There are limited data on the phenotype of myeloid cells in patients with germline loss-of-function mutations in *BTK*.[2] Our detailed immunological study provides evidence of a non-inflammatory profile of myeloid cells in XLA that was sustained upon persistent SARS-CoV-2 infection, providing a possible explanation for the protracted course of our case and others previously reported.[1] Patients with inborn errors of immunity offer unique opportunities to better understand the host-pathogen interactions, despite the limitations imposed by their rarity. The ability to limit the disease severity in this patient with acknowledged risk factors, adds evidence in favor of early use of BTK inhibitors to treat COVID-19, as a strategy to ameliorate the hyper-inflammatory response, improve survival and limit inflammatory complications.

AUTHOR CONTRIBUTIONS

Amelia C. Trombetta, Ana E. Sousa, and Susana L. Silva designed and supervised the study. Inês Parreira, Hélder Diogo Gonçalves, Mariana Lessa Simões, Patrício Aguiar, Maria Manuel Deveza, João Inácio, and Susana L. Silva collected clinical data. André M. C. Gomes, Guilherme B. Farias, Amelia C. Trombetta, and Ana Godinho-Santos performed the experiments. André M. C. Gomes, Guilherme B. Farias, Ana E. Sousa, and Susana L. Silva wrote the paper.

ACKNOWLEDGMENTS

The authors would like to thank all the participating patients and the health care professionals involved in patient care, particularly Susana M. Fernandes, Catarina Mota, Maria Adão-Serrano, Renato Costa-Reis, Sandra Braz, and Teresa Meira. They acknowledge Instituto Português do Sangue e da Transplantação (IPST) and Marc Veldhoen for assistance in convalescent plasma treatment. They also acknowledge Carolina M. Conceição, Joel Laia, Pedro Rosmaninho, Diana F. Santos, Catarina Mota, and Afonso R. M. Almeida for their contribution for the experimental work, as well as the team members of the Flow Cytometry facility at Instituto de Medicina Molecular João Lobo Antunes.

FUNDING INFORMATION

This work was funded by the following grants from Fundação para a Ciência e a Tecnologia (FCT), Portugal, through "Apoio Especial Research4COVID-19," project numbers 125 and 803. André M. C. Gomes and Guilherme B. Farias received Fellowships funded by FCT (Doctorates4COVID-19, 2020.10202.BD), and Janssen-Cilag Farmacêutica, respectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request to the corresponding author.

ORCID

André M. C. Gomes https://orcid.org/0000-0002-5806-4707
Guilherme B. Farias https://orcid.org/0000-0002-9073-9401
Amelia C. Trombetta https://orcid.org/0000-0003-3285-3086
Ana Godinho-Santos https://orcid.org/0000-0001-8555-0789
Inês Parreira https://orcid.org/0000-0003-2017-2697
Hélder Diogo Gonçalves https://orcid.org/0000-0003-4908-2986
Mariana Lessa Simões https://orcid.org/0000-0001-8031-7371
Patrício Aguiar https://orcid.org/0000-0003-2393-3463
Maria Manuel Deveza https://orcid.org/0000-0002-7967-9559
João Inácio https://orcid.org/0000-0003-3210-3285
Ana E. Sousa https://orcid.org/0000-0002-4618-9799
Susana Lopes da Silva https://orcid.org/0000-0003-3943-1185

REFERENCES

1. Ponsford MJ, Shillitoe BMJ, Humphreys IR, Gennery AR, Jolles S. COVID-19 and X-linked agammaglobulinemia (XLA) - insights from a monogenic antibody deficiency. *Curr Opin Allergy Clin Immunol*. 2021;21:525-534.
2. Marron TU, Martinez-Gallo M, Yu JE, Cunningham-Rundles C. Toll-like receptor 4-, 7-, and 8-activated myeloid cells from patients with X-linked agammaglobulinemia produce enhanced inflammatory cytokines. *J Allergy Clin Immunol*. 2012;129:184-190.
3. Trombetta AC, Farias GB, Gomes AMC, et al. Severe COVID-19 recovery is associated with timely Acquisition of a Myeloid Cell Immune-Regulatory Phenotype. *Front Immunol*. 2021;12:691725.
4. Stack M, Sacco K, Castagnoli R, Livinski AA, Notarangelo LD, Lionakis MS. BTK inhibitors for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): a systematic review. *Clin Immunol*. 2021;230:108816.
5. Farias GB, Badura R, Conceição CM, et al. Acute HIV-1 and SARS-CoV-2 infections share Slan+ monocyte depletion-evidence from an hyperacute HIV-1 case report. *Viruses*. 2021;13:1805.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gomes AMC, Farias GB, Trombetta AC, et al. Phenotype of BTK-lacking myeloid cells during prolonged COVID-19 and upon convalescent plasma. *Eur J Haematol*. 2023;110(2):209-212. doi:10.1111/ejh.13881