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SARS-CoV-2 Infection Drives a Glycan Switch of Peripheral T Cells at Diagnosis

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COVID-19 is a highly selective disease in which SARS-CoV-2 infection can result in different clinical manifestations ranging from asymptomatic/mild to severe disease that requires hospitalization. In this study, we demonstrated that SARS-CoV-2 infection results in a glycosylation reprogramming of circulating lymphocytes at diagnosis. We identified a specific glycosignature of T cells, defined upon SARS-CoV-2 infection and apparently triggered by a serological factor. This specific glycan switch of T cells is detected at diagnosis being more pronounced in asymptomatic patients. We further demonstrated that asymptomatic patients display an increased expression of a viral-sensing receptor through the upregulation of DC-SIGN in monocytes. We showed that higher levels of DC-SIGN in monocytes at diagnosis correlates with better COVID-19 prognosis. This new evidence pave the way to the identification of a novel glycan-based response in T cells that may confer protection against SARS-CoV-2 infection in asymptomatic patients, highlighting a novel prognostic biomarker and potential therapeutic target. ***The Journal of Immunology*****, 2021, 207: 1591–1598.**

Coronavirus SARS-CoV-2 is the etiologic agent responsible for the global pandemic of coronavirus disease 2019 (COVID-19), that, as of February 9, has infected (IF) over 106 million people worldwide (1, 2). The overall mortality of COVID-19 is between 0.5 and 3.5% (1, 2). COVID-19 is a highly selective disease. In fact, only some IF individuals get sick, and although most of the critically ill are elderly, some patients that die are previously healthy and/or relatively young (3). There is an urgent need to improve the understanding of the pathophysiology of this disease, envisioning better management in terms of patient care, treatment options, vaccination strategies, and the allocation of health care resources. Vaccine development against SARS-CoV-2 poses a great promise for the resolution of the pandemic, and several vaccines are now being distributed among the world population, namely mRNA- and protein-based approaches (4, 5). However, its long-term protection, effectiveness to reinfection, efficacy to new variants, and availability for the general public is still on trial. This lack of knowledge highlights the importance of understanding patient-specific immune response to infection, envisioning the identification of novel mechanisms of disease. Moreover, comprehensive insights on COVID-19 molecular mechanism may be translated into diagnostic or prognostic biomarkers, therapeutic targets to improve patient's clinical management, and patients stratification for vaccination.

SARS-CoV-2 infection results in a broad range of symptoms (6). Several studies on immune profiling of IF patients consistently revealed an immunological dysregulation, observed in PBMCs. A decrease of T cells and dendritic cells frequencies and an increase of monocytes and neutrophils have been observed in hospitalized IF individuals, as well as a cytokine dysregulation, the so-called "cytokine storm" associated with critical illness (7–10). However, the mechanism underlying this immunological dysregulation remains largely unknown.

Despite being overlooked, the field of glycobiology can provide missing answers to immunological questions. Glycosylation is a major posttranslational mechanism characterized by the enzymatic addition of glycans (carbohydrates) to proteins or lipids of essentially all cells, including immune cells. In fact, glycans are master regulators of immune cell functions, defining the activation and differentiation of T cells (11, 12). In the cellular immune system, we and others have previously demonstrated the regulatory power of

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I.A., M.M.V., and S.S.P. designed research. I.A., M.M.V., J.G., Â.F., A.M.D., and C.S.R. performed research. S.S.P. contributed new reagents/analytical tools. J.C.O., N.S., L.M., M.A.A., and R.S.eC. provided and characterized clinical samples. I.A. and M.M.V. analyzed data. I.A., M.M.V., and S.S.P. wrote the manuscript with contributions from all authors.

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Abbreviations used in this article: AAL, *Aleuria aurantia* lectin; CHUP, Centro Hospitalar e Universitário do Porto; CHVNG, Centro Hospitalar de Vila Nova de Gaia/Espinho; COVID-19, coronavirus disease 2019; DC-SIGN, dendritic cell–specific ICAM-3–grabbing nonintegrin; GNA, *Galanthus nivalis* lectin; IF, infected; L-PHA, *Phaseolus vulgaris* leucoagglutinin; SNA, *Sambucus nigra* lectin.

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glycans in adaptive immune response through the modulation of T cell activation thresholds associated with the immunopathogenesis of autoimmune diseases and cancer (13–19). Moreover, being present in several pathogens, glycans can also be sensed by immune cells through their recognition by specific glycan-recognizing receptors expressed in those cells (20, 21). Like the majority of viruses, SARS-CoV-2 viral capsids are also covered with glycans. More specifically, the spike protein of the SARS-CoV-2 envelope was shown to be highly glycosylated (harboring 22 *N*-glycosylation sites) with glycan structures such as oligomannose and some branched *N*-glycans (22, 23). These structures can be specifically recognized by glycan binding proteins of the host's immune cells, such as the C-type lectin dendritic cell–specific ICAM-3–grabbing nonintegrin (DC-SIGN), present in innate immune cells, promoting virus recognition and elimination (24, Amraei, R., W. Yin, M. A. Napoleon, E. L. Suder. J. Berrigan, Q. Zhao, J. Olejnik, K. B. Chandler, C. Xia, J. Feldman, et al., manuscript posted on medRxiv, DOI: 10.1101/2020.06.22.165803, and Hoffmann, D., S. Mereiter, Y. Jin Oh, V. Monteil, R. Zhu, D. Canena, L. Hain, E. Laurent. C. Grünwald-Gruber, M. Novatchkova, et al., manuscript posted on medRxiv, DOI: 10.1101/2021.04.01.438087).

Defining the glycosylation profiles of immune cells in SARS-CoV-2–IF individuals and how they impact their effector functions remains completely unknown, but it is of the utmost importance for the understanding of COVID-19 immunopathogenesis as well as for the improvement of the clinical management of the disease and for pandemic control measures, namely as a stratification biomarker.

In this study, we discovered that circulating T cells exhibit a glycan switch upon SARS-CoV-2 infection that is detected at COVID-19 diagnosis. This change in the glycosylation profile of T cells appears to be triggered by a serum inflammatory factor present in IF patients. This specific T cell glycan switch is more pronounced in asymptomatic patients rather than in those who exhibit symptoms. Importantly, from a clinical standpoint, we also unveiled that COVID-19 patients with "good prognosis" exhibit an upregulation of DC-SIGN expression in circulating monocytes.

Materials and Methods

Cohort description and patient's selection criteria

The present study integrates a total of 32 patients diagnosed with SARS-CoV-2 from two individual Portuguese cohorts (between May 2020 and July 2020), 20 patients from the Infectious Disease Department of Centro Hospitalar e Universitário do Porto (CHUP) (Porto, Portugal), and 12 patients from the Infectious Disease Department of Centro Hospitalar de Vila Nova de Gaia/Espinho (CHVNG) (Vila Nova de Gaia, Portugal). The total cohort includes patients of both genders and age between 22- and 89-y-old. All the samples were included in all the FACS analysis discussed.

Blood was collected, and plasma and PBMCs were isolated at the time of diagnosis, within the first 72 h of PCR^+ SARS-CoV-2 test, and 14 d ($n = 2$) after diagnosis. Similar analysis was conducted in a subset of patients ($n = 2$) that recovered.

The eligibility criteria for inclusion in this study were SARS-CoV-2–positive patients asymptomatic or symptomatic with different levels of severity (mild, moderate, and severe). Asymptomatic patients ($n = 5$) were defined as positive for the SARS-CoV-2 PCR test but no signs of diseases. The following criteria were used to stratify symptomatic SARS-CoV-2 patients in terms of the disease severity and accordingly with World Health Organization guidelines (25): 1, mild ($n = 18$): individuals with no evidence of pneumonia; 2, moderate ($n = 6$): individuals without need of invasive mechanical ventilation and without need of admission to hospital intensive care unit, but there is evidence of pneumonia and need of supplementary oxygen; and 3, severe ($n = 1$): individuals with need of invasive mechanical ventilation and with need of admission to hospital intensive care unit. Severity was determined both at diagnosis and 14 d postdiagnosis. Patient demographics and relevant clinical data are summarized in Supplemental Table I.

Healthy controls ($n = 5$) are represented by volunteer individuals with no history of infection disorders that went to CHUP for routine analysis. Additionally, to evaluate the predictive value of DC-SIGN expression, patients were grouped according to disease severity at day 14 postdiagnosis. Patients were then classified as having a good prognosis when they were asymptomatic at diagnosis and remained without symptoms throughout the follow-up and until day 14 postdiagnosis, as well as symptomatic patients at diagnosis that improved over the disease course, alleviating symptoms at day 14 postdiagnosis. Patients that either did not improve symptoms and disease severity or aggravated symptoms by displaying worsened disease at day 14 postdiagnosis were classified as "poor prognosis."

All participants gave informed consent about all clinical procedures, and research protocols were approved by the ethics committee of CHUP and CHVNG in Portugal.

Human PBMCs isolation

Human PBMCs from COVID-19 patients and healthy donors were isolated by gradient centrifugation using 1 vol of Lymphoprep (STEMCELL Technologies) for 2 vol of blood for 30 min at 900 × *g* with the brake off. The upper phase containing the serum was collected and stored at −80°C. PBMCs (interphase) were collected, washed twice with PBS, and incubated with FVD–allophycocyanin-eFluor780 (eBioscience) for 30 min. Cells were washed with PBS, fixed with 2% formaldehyde (PanReac AppliChem), and resuspended in FACS buffer (PBS 2% FBS). All procedures were performed under biosafety level 3 conditions.

Flow cytometry staining

For lectin staining, cells were incubated with conjugated lectins (Vector Laboratories): *Phaseolus vulgaris* leucoagglutinin (L-PHA-fluorescein, FITC), *Galanthus nivalis* lectin (GNA-fluorescein, FITC), biotinylated *Sambucus nigra* lectin (SNA), and biotinylated *Aleuria aurantia* lectin (AAL) for 15 min. Biotinylated lectins were incubated with streptavidin-PE-Cy7 (eBioscience) for 30 min. For surface marker staining, cells were stained for 30 min on ice while protected from light with the following Abs: allophycocyanin anti-human TCRγδ (clone B1), BV510 anti-human CD69 (clone FN50), PE anti-human CD25 (clone BC96), BV421 anti-human CD14 (clone 63D3), PE anti-human CD56 (clone HCD56), and PE-Cy7 anti-human CD206 (clone 15-2) from BioLegend; eFluor 450 anti-human CD4 (clone RPA-T4), PerCP-eFluor 710 anti-human PD-1 (clone J105), PE-Cy5 anti-human CD19 (clone HIB19), eFluor 450 anti-human IgM (clone SA-DA4), eFluor 660 anti-human galectin 3 (clone M3/38), allophycocyanin anti-human CD11c (clone BU15), and PE-Cy5 anti-human CD86 (clone IT2.2) from eBioscience; PE anti-human TCRα/β (clone BW242/412) from Miltenyi Biotec; and BV510 anti-human CD3 (clone OKT3) from BD Biosciences. For DC-SIGN staining, cells were incubated with DC-SIGN rabbit IgG (Bio-Rad Laboratories) followed by incubation with polyclonal swine anti-rabbit IgG (FITC; Dako) for 30 min on ice. Cells were resuspended in FACS buffer prior to analysis. Data were obtained on a BD FACS Canto II instrument (Becton Dickinson) and analyzed using FlowJo v10.0 (Tree Star).

IL-2 serum quantification

Plasma from four non-IF and eight COVID-19 patients (four asymptomatic and four moderate disease patients) were analyzed by flow cytometry using the BD Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit (BD Biosciences), following the manufacturer's instructions.

In vitro assessment of glycan modulation of T cells

PBMCs from healthy and independent from the COVID-19 cohort human donors were isolated from fresh collected blood, as described above. PBMCs were cultured in RPMI-1640 (Life Technologies) supplemented with 10% (v/v) heat-inactivated FBS, 1% penicillin/streptomycin, and 10% of plasma from four asymptomatic or moderate patients for each condition. Different cell culture conditions were tested, namely human plasma concentration (25 and 10%) as well as time of culture (4 d, 2 d, 24 h, and 12 h). The optimal culture condition (with lower cell death and stronger glycan modulation) was selected (10% human plasma and 24 h cell culture). After 24 h, cell glycoprofile was evaluated using the same Abs and lectin panel selected above. Serum IL-2 was neutralized by incubating PBMCs from healthy donors with 20 μg/ml of anti–IL-2 (5334, R&D Systems) and 10% serum from non-IF and COVID-19 patients (four asymptomatic and four moderate patients) for 24 h. After 24 h, the cellular glycoprofile was evaluated as above. Data were obtained on a BD FACS Canto II instrument (Becton Dickinson) and analyzed using the FlowJo 10.0 software (Tree Star).

Data visualization and statistical analysis

Data visualization and statistical analyses (nonparametric Mann–Whitney *t* test) were done using GraphPad Prism 9 software.

The prediction capacity of DC-SIGN levels to discriminate patients who develop a poor disease course from those that have a good disease course

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was determined by plotting the receiver operating characteristic curves and calculating the area under the curve. The cut-off that revealed the best balance between sensitivity and specificity was selected for the subsequent statistical analysis. Sensitivity, specificity, and positive and negative predictive values were calculated. Univariate binary logistic regression analysis was performed to test DC-SIGN levels and disease progression (good: asymptomatic maintained asymptomatic and/or severity decreased at least one category; poor: symptoms were not improved and/or escalate to a higher severity). In logistic regression, model goodness-of-fit was assessed by the Hosmer–Lemeshow statistical test. Results are presented as odds ratios for each category as compared with a predefined reference category and their respective 95% confidence intervals. Odds ratios above one and below one are indicative, respectively, of higher and lower odds of developing a poor disease course as compared with a reference category. Statistical analysis was performed using the statistical software SPSS version 25 (IBM, IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY; released 2017) The threshold used for statistical significance was p value < 0.05.

Results

SARS-CoV-2–IF individuals display decreased β1,6-GlcNAc–branched and α2,6-sialic acid N*-glycans on peripheral T cells at diagnosis*

Taking into consideration the fact that the underlying mechanisms that explain a differential interindividual clinical presentation of COVID-19 at diagnosis are still unclear, we, in this study, characterized the glycosylation profile of T cells from patients' peripheral blood. A lectin-based flow cytometry of the T cell populations was performed to evaluate glycosylation profiles (gating strategy in Supplemental Fig. 1A). We started by analyzing the levels of the expression of β1,6-GlcNAc–branched *N*-glycan structures, known to have a major impact on the regulation of T cell activity and function (13, 16, 26), using the L-PHA lectin. Our results demonstrated that, overall, T cells from IF subjects have a decrease in branched *N*-glycan structures, particularly on $CD8^+$ and γδ T cells, when compared with non-IF ones (Fig. 1A, top and bottom left panel). Moreover, T cells from asymptomatic patients have lower levels of β1,6-GlcNAc–branched *N*-glycans that gradually tend to increase along disease severity (Fig. 1A, right). Notably, $CD8^+$ T cells display a significant decrease in the levels of the expression of complex branched *N*-glycans in asymptomatic and mild disease patients when compared with non-IF individuals (Fig. 1A, top).

Concerning α2,6-sialylation (recognized by the SNA lectin), already described to play a role in the regulation of T cell immune response (27), our results demonstrate that $CD8^+$ T cells from IF patients display a significantly decreased SNA binding, in comparison with non-IF individuals (Fig. 1B, top left). Consistently, asymptomatic patients present the lowest levels of SNA binding compared with non-IF (Fig. 1B, top right). Even though the levels of SNA binding were shown to be associated with age (Supplemental Fig. 1E), there are no significant differences between the mean age of asymptomatic and symptomatic patients (Supplemental Table I). Curiously, for both glycans, the two COVID-19 convalescent patients seem to recover (or surpass) the levels of expression of β1,6-GlcNAc–branched and α2,6-sialic acid glycan structures in all T cell subsets (Fig. 1A, 1B, right). These results showed that $CD8^+$ T cells from asymptomatic patients display a defect in branched and sialylated *N*-glycans when compared with non-IF.

The differences observed in the glycosylation profile of T cells across severity were not related to the abundance or activation of T cell populations because $CD3^+$, $CD4^+$, $CD8^+$, and γδ T cells showed similar frequencies as well as similar levels of PD1 and CD69 expression between the groups, with the exception of lowered $CD3^+$ population and increased $CD69^+$ within $CD8^+$ T cells in the mild disease group (Supplemental Fig. 1A–C).

In addition, because glycosylation is a dynamic process that can change along with disease status, we took advantage of a limited longitudinal follow-up analysis using a single patient, representative of each severity group (asymptomatic, mild, moderate, and severe), which revealed that levels of expression of β1,6-GlcNAc and α2,6-sialylation are restored in patients with improved disease severity at day 14 postdiagnosis (Supplemental Fig. 1D).

No major differences were observed for other glycan structures such as mannosylation (GNA binding) and fucosylation (AAL binding) in the different T cell subsets at diagnosis (Supplemental Fig. 1F, 1G).

These results showed that SARS-CoV-2–IF individuals led to an en bloc decreased expression of specific glycan structures such as β1,6-GlcNAc–branched *N*-glycans and α2,6-sialic acid in T cells, predominately in $CD8^+$ T cells and asymptomatic patients. The absence of differences on high mannose structures (GNA binding) or fucose residues on T cells (AAL binding) supports the specificity of the glycan switch. In fact, decreased levels of β1,6-branched *N*-glycans in T cells have been pointed out as a mechanism of TCR threshold regulation by hampering the TCR complex clustering and lowering the necessary amount of Ag recognition to signal a cellular response (16). Moreover, surface α2,6-sialylation also regulates the T cell immune response through galectins binding modulation (28, 29).

To further clarify the underlying mechanism that imposes the changes in the glycosylation profile of circulating T cells upon SARS-CoV-2 infection, we have performed an in vitro assay in which PBMCs isolated from healthy individuals were incubated with plasma from four patients from either each group: non-IF, asymptomatic, or moderate disease. Our results showed that T cells, upon incubation with plasma from asymptomatic patients, displayed a significant decreased expression of β1,6-GlcNAc–branched *N*-glycans (Fig. 2A) and α2,6-sialylation (Fig. 2B) when compared with T cells cultured with plasma from non-IF individuals and moderate disease patients. These results suggest the existence a specific serum factor that in asymptomatic patients drives a remarkable drop of complex branched and sialylated glycans structures on T cells. In fact, it is known that asymptomatic patients display a differential serum composition of cytokines and chemokines when compared with symptomatic (10, 30, and B. Pérez-Cabezas, R. Ribeiro, I. Costa, S. Esteves, A. R. Teixeira, T. Reis, R. Monteiro, A. Afonso, V. Pinheiro, M. I. Antunes, et al, manuscript posted on medRxiv). Moreover, T cell glycosylation is known to be modulated by specific ILs, namely IL-2 and IL-7, that regulate the transcription of specific Golgi glycosyltransferases (17, 31). In addition, IL-2 was described to be a specific biomarker of SARS-CoV-2 cellular response (B. Pérez-Cabezas, R. Ribeiro, I. Costa, S. Esteves, A. R. Teixeira, T. Reis, R. Monteiro, A. Afonso, V. Pinheiro, M. I. Antunes, et al, manuscript posted on medRxiv). Given that IL-2 has been pointed out to be involved in SARS-CoV-2 immune responses and that its signaling drives a glycan modulation in T cells, we evaluated the levels of this cytokine in the serum of the individuals in our cohort. Interestingly, we found that asymptomatic patients that exhibited decreased levels of branched *N*-glycans on T cells (Fig. 2A) had lower levels of IL-2 in the serum when compared with non-IF and moderate patients (Fig. 2C). Therefore, we hypothesized that levels of IL-2 would be involved in T cell glycan switching. To test this, we set up the same in vitro approach by incubating PBMCs with serum from either asymptomatic or moderate patients, in which IL-2 was inhibited using anti–IL-2–neutralizing Ab. Our results showed that the inhibition of IL-2 resulted in decreased levels of β1,6-GlcNAc branching in both $CD8^+$ and $CD4^+$ T cells (Fig. 2D, 2E). This evidence pinpoint IL-2 as a potential serum

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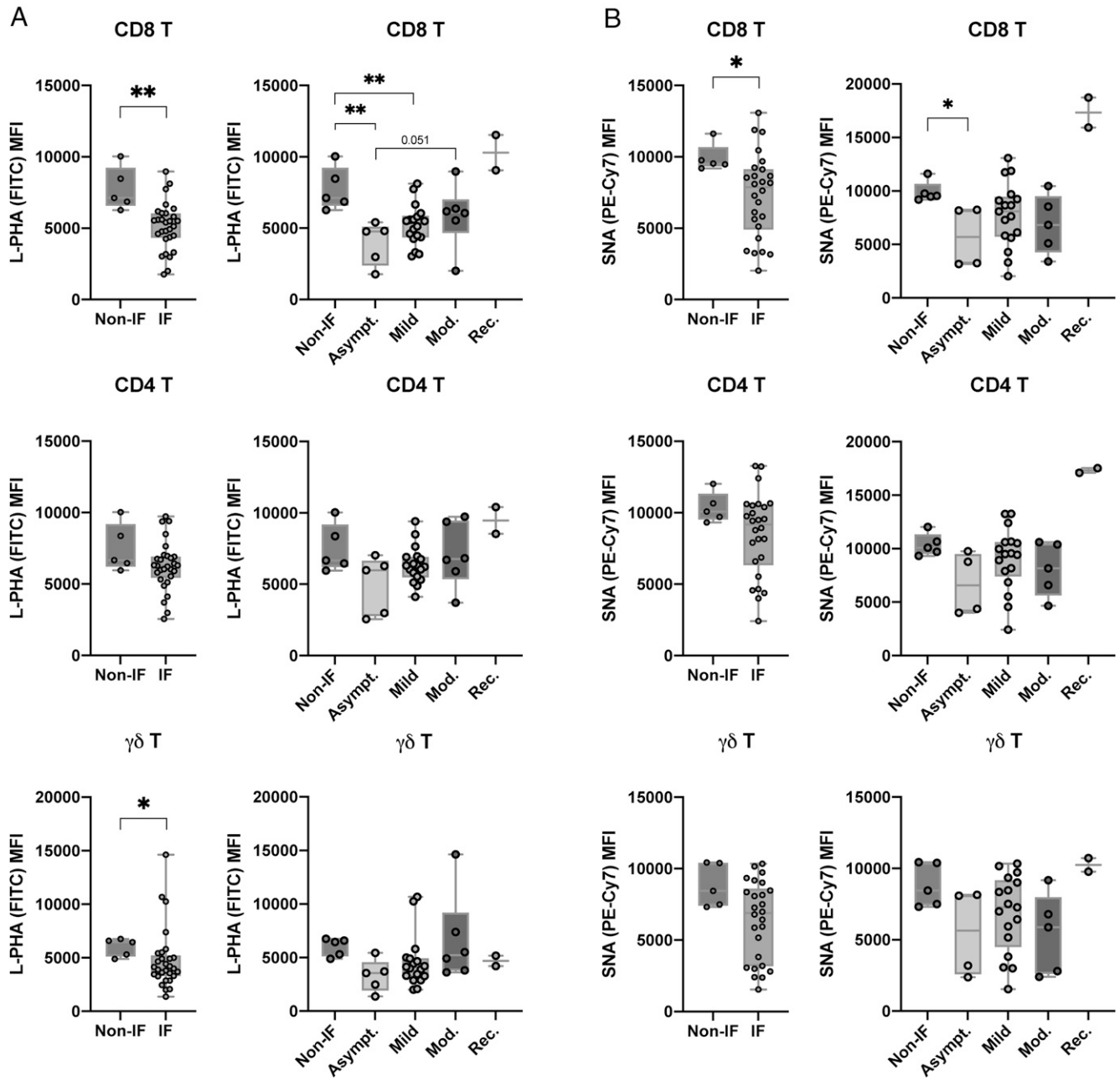

FIGURE 1. Peripheral T cell glycoprofile is altered upon SARS-CoV-2 infection at diagnosis. Levels (mean intensity fluorescence, MFI) of (**A**) L-PHA lectin binding detecting β1,6-GlcNAc–branched *N*-glycans and (**B**) SNA lectin binding detecting α2,6-sialylation in $CD8^+$, $CD4^+$, and γδ T cell subsets of non-IF ($n = 5$) and IF ($n = 30$) patients. Levels of (A) L-PHA and (B) SNA lectin binding for each disease severity group: asymptomatic (Asympt.; $n = 5$), mild ($n = 18$), moderate (Mod.; $n = 6$), and recovered (Rec; $n = 2$). Each dot represents one patient. Mann–Whitney *t* test was performed to evaluate statistically significant differences between each group pair. $*p < 0.05$, $**p < 0.005$.

factor involved in T cells glycan switching, highlighting a promising clinical target for hampering inefficient immune responses through glycosylation remodeling of T cells, controlling severe symptoms observed in COVID-19 patients.

Levels of DC-SIGN expression in circulating monocytes predict COVID-19 prognosis. To gain further insights in the viral–glycan recognition mechanism, we have characterized the expression of specific glycan binding proteins expressed by innate immune cells that are known to sense and recognize specific viral glycans, instructing an immune response (32). Regarding the abundance of innate cells, we have observed an increase in the frequency of dendritic cells as well as monocytes in asymptomatic patients when compared with non-IF individuals, and no differences in terms of NK and NKT cells subsets (Supplemental Fig. 1H, 1I). Regarding their activation state, analyzed by CD86 expression, only monocytes are shown to be activated in asymptomatic and mild disease patients (Supplemental Fig. 1C). This is in accordance with previous findings (33).

Specifically, DC-SIGN, a C-type lectin that recognizes mannose structures, is already known to play a role in the SARS-COV-2 recognition (24, Amraei, R., W. Yin, M. A. Napoleon, E. L. Suder. J. Berrigan, Q. Zhao, J. Olejnik, K. B. Chandler, C. Xia, J. Feldman, et al., manuscript posted on medRxiv, DOI: 10.1101/2020.06.22.165803, and Hoffmann, D., S. Mereiter, Y. Jin Oh, V. Monteil, R. Zhu, D. Canena, L. Hain, E. Laurent. C. Grünwald-Gruber, M. Novatchkova, et al., manuscript posted on medRxiv,

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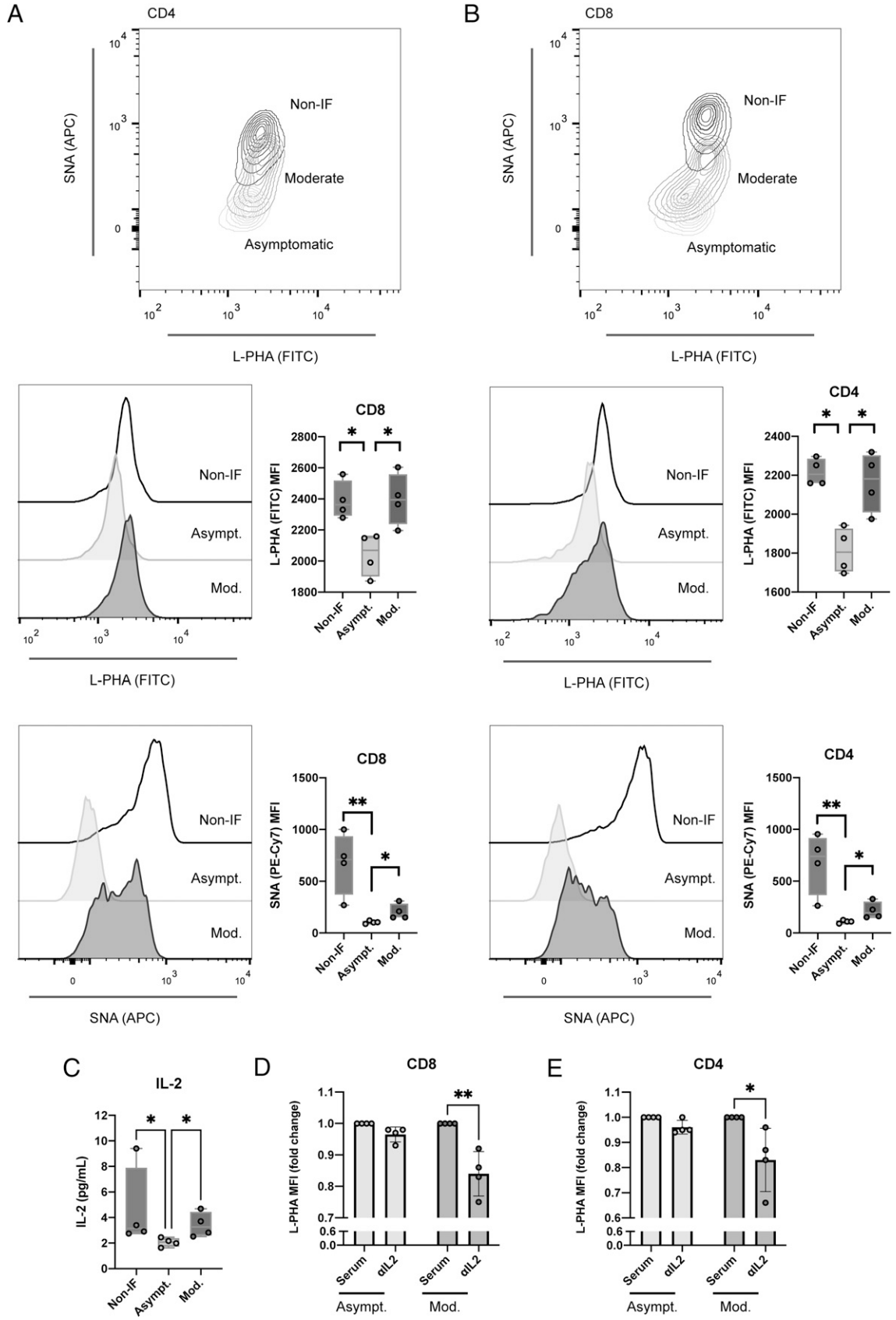

FIGURE 2. Patient-derived serum induces a differential T cell glycosylation comparing asymptomatic and symptomatic patients (**A**) L-PHA lectin and (**B**) SNA lectin binding (mean fluorescence intensity, MFI) of $CD8^+$ and $CD4^+$ T cells from a healthy donor, independent of our cohort, incubated for 24 h with 10% plasma from four patients from each group (non-IF, asymptomatic, or moderate). (**C**) Plasma levels of IL-2 from four patients from each group (non-IF, asymptomatic, or moderate). (**D**) Effect of neutralizing anti–IL-2 on the L-PHA levels of $CD4^+$ and (**E**) $CD8^+$ T cells in asymptomatic and moderate patients. Each dot represents a patient. Gating strategies for each population are included in Supplemental Fig. 1A. Mann–Whitney *t* test was performed to evaluate statistically significant differences between each group pair. $*p < 0.05$, $**p < 0.005$.

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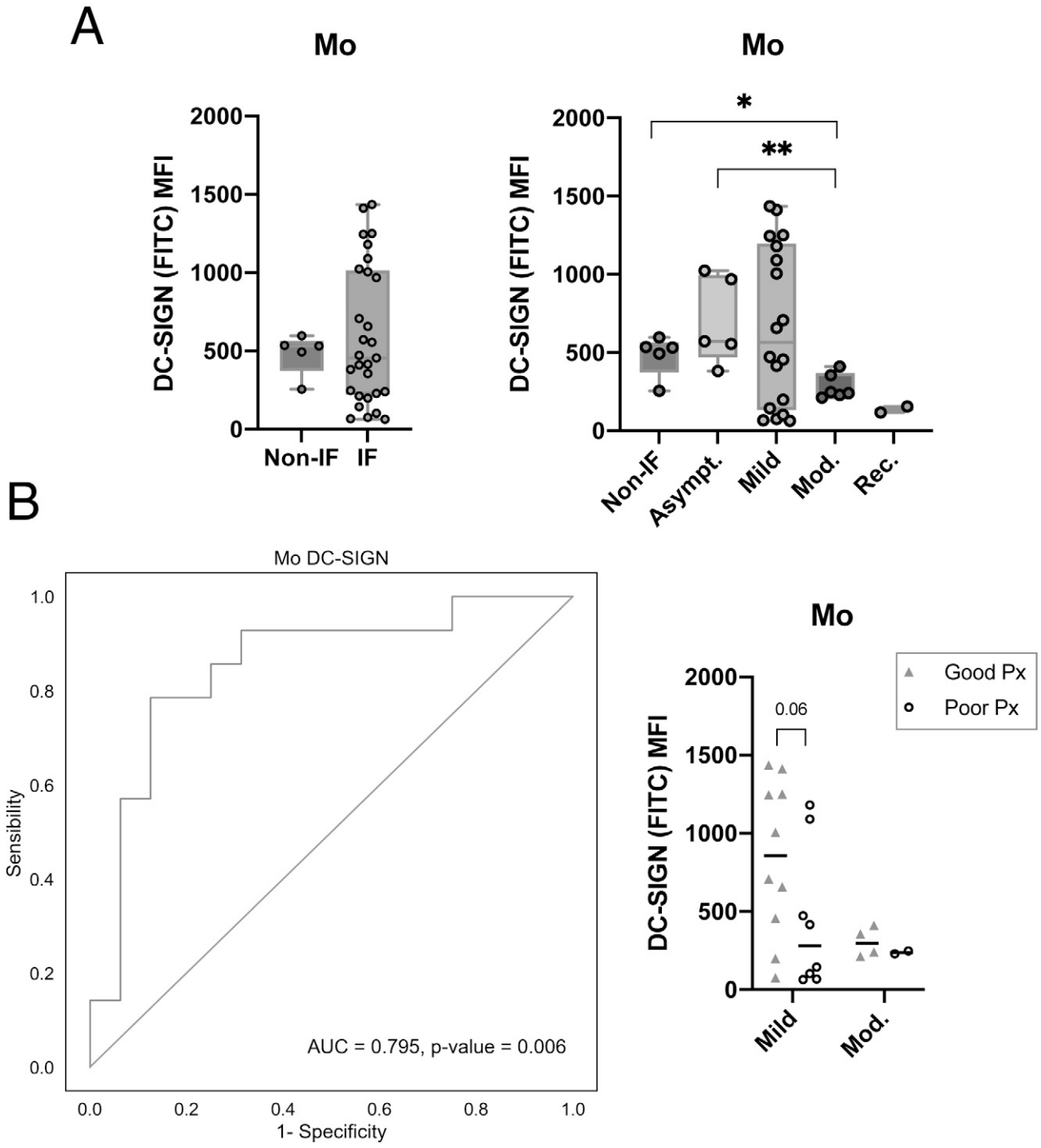

FIGURE 3. DC-SIGN expression in peripheral monocytes as a predictor of good versus poor prognosis in COVID-19 patients. (**A**) Levels of DC-SIGN surface expression (mean fluorescence intensity, MFI) in monocytes (Mo). (**B**) Receiver operating characteristic curve plotted for the DC-SIGN expression levels in monocytes from COVID-19 patients. The distribution of DC-SIGN levels are represented regarding the prognosis (Px) (day 14; good Px in green and poor Px in gray) as well as the severity at diagnosis. Each dot represents one patient; $n = 24$. Gating strategies for each population are included in Supplemental Fig. 1A. Mann–Whitney t test was performed to evaluate statistically significant differences. $^*p < 0.05$, $^{**}p < 0.005$, or represented.

DOI: 10.1101/2021.04.01.438087), being expressed by monocytes, immature dendritic cells, and macrophages. Our results showed that patients from the asymptomatic group displayed higher levels of DC-SIGN expression in monocytes compared with moderate disease (Fig. 3A), suggesting an increased capacity of asymptomatic patients to sense and recognize the virus.

Taking into consideration the biological relevance of DC-SIGN expression in monocytes as the first sensing mechanism upon SARS-CoV-2 infection and given the heterogenous expression of DC-SIGN among IF individuals (at diagnosis) (Fig. 3A), we next analyzed its prognostic value. A longitudinal cohort was used with clinical information in terms of disease progression (development of a poor versus good prognosis at day 14 postdiagnosis, characterized in Supplemental Table I). The symptoms and disease severity at day 14 postdiagnosis were considered as criteria to define the prognosis. Patients with good prognosis were those individuals that either remained asymptomatic or improved symptoms/disease severity between diagnosis and day 14 postdiagnosis. The poor prognosis

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group includes patients that did not improve across disease course or displayed a worsened disease and aggravated symptoms at day 14 postdiagnosis. Remarkably, the expression levels of DC-SIGN in monocytes were found to be able to stratify patients at diagnosis in terms of their likelihood of developing a good versus a poor disease prognosis with a specificity and sensitivity of 78.9% and 63.6%, respectively (Fig. 3B). The univariate analysis demonstrated that high levels of DC-SIGN expression in monocytes at diagnosis increase the odds ratio associated with the prediction of having a good prognosis (odds ratio = 6.548; $p = 0.011$). To assess the influence of cellular activation in DC-SIGN upregulation, we have evaluated the correlation between DC-SIGN expression levels and CD86 in monocytes, and no correlation was observed (Supplemental Fig. 1J, 1K). Moreover, taking into consideration that the presence of comorbidities has been associated with COVID-19 prognosis, as also observed in our cohort (Supplemental Fig. 1L), we evaluated whether the presence of comorbidities would influence DC-SIGN expression, and the results showed no significant association between DC-SIGN levels and the presence of comorbidities (Supplemental Fig. 1M), which excludes this variable as a potential contributor for the different DC-SIGN expression in monocytes among these patients. In fact, the recognition of the coronavirus glycans by innate immune cells was demonstrated to be mediated by DC-SIGN (24, Amraei, R., W. Yin, M. A. Napoleon, E. L. Suder. J. Berrigan, Q. Zhao, J. Olejnik, K. B. Chandler, C. Xia, J. Feldman, et al., manuscript posted on medRxiv, DOI: 10.1101/2020.06.22.165803, and Hoffmann, D., S. Mereiter, Y. Jin Oh, V. Monteil, R. Zhu, D. Canena, L. Hain, E. Laurent. C. Grünwald-Gruber, M. Novatchkova, et al., manuscript posted on medRxiv, DOI: 10.1101/2021.04.01.438087). An increased expression of this C-type lectin at the initial days of infection suggests a more efficient viral recognition and subsequent clearance, resulting in a good prognosis of the disease.

Discussion

Our results demonstrated for the first time, to our knowledge, that SARS-CoV-2 infection imposes a glycosylation reprogramming of adaptive immune cells, suggesting a specific glycan switch of T cells that may define their ability to successfully deal with infection. We identified a specific glycosylation signature of circulating T cells that is associated with their activity and function, distinguishing IF patients from non-IF individuals. Our results also suggest that the glycan switch imprinted in circulating T cells appear to be mediated by a serum extracellular factor(s), pinpointing IL-2 as a potential driver of T cells glycan switching. In fact, previous evidence from others and us demonstrated that the deficiency in branched *N*-glycan structures on T cells imposes a hyperreactive phenotype with a decreased threshold of T cell activation and increased T cell activity (13, 16). We, in this study, propose that the immune response against SARS-CoV-2 infection appears to be influenced by the glycosylation profile of circulating T cells, which defines their effective functions. In fact, a pronounced deficiency of complex branched and sialylated glycans on $CD8^+$ and γδ T cells were observed in asymptomatic COVID-19 patients. This dynamic and plastic glycoimmune modulation ("Glyco-Immune Alert" mechanism) may constitute a novel mechanism of host response, which contributes to further understanding the immunological differences among IF individuals (Supplemental Fig. 2).

Furthermore, we also showed another glycan-based mechanism of host response to SARS-CoV-2 driven by the upregulation of the viral glycans recognizing receptor DC-SIGN in monocytes, with prognostic application when detected at diagnosis.

This study unlocks the identification of a specific glycosignature of T cells as well as a prognostic biomarker in COVID-19. Further studies are needed to explore the cellular and molecular mechanisms underlying IL-2–mediated T cell glycan switch in infection and along disease course. This knowledge will be fundamental to pinpoint IL-2 as a critical serological factor that instructs the glycoreprogramming of peripheral T cells, envisioning the identification of a potential novel biomarker and therapeutic target in COVID-19. Our study was conducted during the first wave of COVID-19 (March–July 2020) and had a limitation in terms of sample size, indicating the need to validate these promising observations in larger and well-characterized multicentric cohorts as well as analyzing the impact of other SARS-CoV-2 variants in T cell glycan switch.

This new evidence in COVID-19 paves the way to the identification of a specific blood glycosignature that is able to stratify patients at diagnosis according with their risk to evolve to worsened disease. This will certainly contribute to improved vaccination strategy and patient risk stratification, optimizing an effective allocation and management of health care resources such as ventilators and intensive care facilities.

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Disclosures

The authors have no financial conflicts of interest.

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