Current Research in Microbial Sciences 6 (2024) 100215

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Persistence of circulating CD169+monocytes and HLA-DR downregulation underline the immune response impairment in PASC individuals: the potential contribution of different COVID-19 pandemic waves

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ARTICLE INFO

Keywords:
CD169
COVID-19 waves
HLA-DR
PASC
Systemic immune-inflammation index

ABSTRACT

The use of CD169 as a marker of viral infection has been widely discussed in the context of COVID-19, and in particular, its crucial role in the early detection of SARS-CoV-2 infection and its association with the severity and clinical outcome of COVID-19 were demonstrated. COVID-19 patients show relevant systemic alteration and immunological dysfunction that persists in individuals with post-acute sequelae of SARS-CoV-2 infection (PASC). It is critical to implement the characterization of the disease, focusing also on the possible impact of the different COVID-19 waves and the consequent effects found after infection. On this basis, we evaluated by flow cytometry the expression of CD169 and HLA-DR on monocytes from COVID-19 patients and PASC individuals to better elucidate their involvement in immunological dysfunction, also evaluating the possible impact of different pandemic waves. The results confirm CD169 RMFI is a good marker of viral infection. Moreover, COVID-19 patients and PASC individuals showed high percentage of CD169+ monocytes, but low percentage of HLA-DR+ monocytes and the alteration of systemic inflammatory indices. We have also observed alterations of CD169 and HLA-DR expression and indices of inflammation upon different COVID-19 waves. The persistence of specific myeloid subpopulations suggests a role of CD169+ monocytes and HLA-DR in COVID-19 disease and chronic post-infection inflammation, opening new opportunities to evaluate the impact of specific pandemic waves on the immune response impairment and systemic alterations with the perspective to provide new tools to monitoring new variants and diseases associated to emerging respiratory viruses.

Introduction

After more than three years of pandemic, on 5 May 2023, the WHO officially declared the end of the COVID-19 emergency, which caused nearly 0.7 billion of cases around the world and fatal outcome in about 7 million individuals (WHO, 2023). Due to the airborne spread, SARS-CoV-2 quickly disseminated increasing its variability and generating several variants. Through genome sequencing, it has been possible to study and monitor the evolution and associated spread of variants (Campbell et al., 2021; Hodcroft et al., 2021), but also ensure the formulation of a specific vaccine (Kyriakidis et al., 2021). During the course of the COVID-19 pandemic, the SARS-CoV-2 virus, particularly at the level of the spike gene, acquired numerous mutations that generated

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https://doi.org/10.1016/j.crmicr.2023.100215

Available online 12 December 2023

Abbreviations

Centers for Disease Control and Prevention CDC
Charlson Comorbidity Index CCI
dendritic cells DC
healthy donors HD
human immunodeficiency virus HIV
human leukocyte antigen-DR HLA-DR
International Classification of Diseases, Tenth Edition Clinical Modification ICD-10-CM
lactate dehydrogenase LDH
mean fluorescence intensity MFI
Neutrophil to lymphocyte ratio NLR
patients positive for SARS-CoV-2 COV
Platelet-to-Lymphocyte Ratio PLR
Policlinico "Tor Vergata" PTV
polymerase chain reaction PCR
post-acute sequelae of SARS-CoV-2 infection PASC
ratio of the mean fluorescence intensity RMFI
Reactive C Protein RCP
sialic acid binding immunoglobulin-like lectin SIGLEC
Systemic immune inflammation index SII
World Health Organization WHO

the spread of different defined variants characterizing the outbreak of different COVID-19 waves (Aleem et al., 2023; Markov et al., 2023). In Italy, several COVID-19 waves characterized by prevalent variants have been defined: the first (March 2020-June 2020) and second (September 2020-January 2021) waves with prevalence of SARS-CoV-2 variants defined as non-VOCs, the third wave (February 2021-June 2021) characterized by the alpha variant of SARS-CoV-2, the fourth wave (July 2021-September 2021) with delta variant, and finally the fifth wave (October 2021-February 2022) with prevalence of delta and omicron variants (Ferrante, 2022; Reno et al., 2022; Boriani et al., 2023).

Considering the multiple variants and different waves, COVID-19 includes many alterations and symptoms. The most common symptoms found in COVID-19 patients were upper and lower respiratory, systemic, gastrointestinal, olfactory and taste, and neurological. However, the type of symptom, severity, duration, and time of onset varied from patient to patient, thus defining a heterogeneity (Ochani et al., 2021). The pathological picture of patients with COVID-19 is denoted by significant hyperinflammation associated with the cytokine storm and the subsequent immunological dysfunction (Sadeghi et al., 2021). Interestingly, the IFN-mediated response was found to be critical during the early phase of SARS-CoV-2 infection and was shown to be closely involved in the evolution of the disease. Indeed, patients with severe COVID-19 were shown to have mutations in genes involved in the regulation of type I and III immunity (Zhang et al., 2020). Moreover, viral infections induced the type I IFNs production that modulate, among others, the expression of an adhesion molecule known as sialic acid binding immunoglobulin-like lectin 1 (SIGLEC1)/CD169 that plays an important role in the host-microbe cross-talk in viral infections, including those caused by the Ebola virus and human immunodeficiency virus (HIV) (Puryear et al., 2013). CD169 has been shown to be expressed on the surface of dendritic cells (DC) and monocytes after the release of antiviral molecules. The CD169 expression in monocytes has been indeed correlated with IFN type I levels (Pino et al., 2015; Bourgoin et al., 2020a).

The utility of CD169 as a biomarker in SARS-CoV-2 infection has been documented (Bourgoin et al., 2020b; Comins-Boo et al., 2021; Herzog et al., 2022). In our previous work, we have used a novel biomarker of viral infections defined as the ratio (R) of CD169 median fluorescence intensity (MFI) between activated monocytes and lymphocytes (CD169 RMFI), demonstrating the correlation of the CD169 RMFI with senescence and exhaustion of the CD8 T cell subset in COVID-19 patients, as well as with markers of maturation and differentiation. Notably, at early infection CD169 RMFI also reflected the disease severity and predicted the respiratory outcome of COVID-19 patients during hospitalization. Furthermore, in vitro stimulation with the SARS-CoV-2 spike protein was found to be able to trigger CD169 in PBMCs in a dose-dependent manner, in association with increased transcription of the IL-6 and IL-10 genes (Minutolo et al., 2021).

Considering its ability to mediate the innate and adaptive response, CD169 might, play a role as a sentinel and potentially in association with post-infection status (Grabowska et al., 2018; Camara et al., 2022). The impact of COVID-19 was protracted in individuals who experienced persistent symptoms at the end of SARS-CoV-2 infection. Complex symptoms lasting four or more weeks after infection have been defined by the Centers for Disease Control and Prevention (CDC) as post-COVID conditions (PCC) or post-acute sequelae of SARS-CoV-2 infection (PASC), or long COVID, characterized by physical, social, and psychological impairments (Chevinsky et al., 2021; CDC, 2022; O'Laughlin et al., 2022). The PASC condition was incorporated into the International Classification of Diseases, Tenth Edition Clinical Modification (ICD-10-CM), published by the World Health Organization (WHO, 2021) and are defined by different and heterogeneous symptoms that can appear together or alone: neurological, psychiatric, skin, gastrointestinal, systemic, and cardiorespiratory (Carfì et al., 2020; Peluso and Deeks, 2022; Soriano et al., 2022). In PASC, the persistence of inflammatory cytokines such as IL-1β, IL-6, TNFα, and also low levels of IL-4, IL-10 (Holms, 2022; Queiroz et al., 2022) in serum has recently been demonstrated. Furthermore, persistent dysregulation of immune cell subtypes was found in PASC up to 24 weeks after acute infection (Ryan et al., 2022), although no evidence of active virus replication.

Considering the impact of COVID-19 even in the long term, as found in PASC, it is necessary to implement the characterization of the disease, focusing also on the possible impact of the different COVID-19 waves and the consequent effects found after infection. To this purpose the aim of the study was to investigate the expression of CD169 and HLA-DR on monocytes from COVID-19 patients and PASC individuals by flow cytometry according to the related pandemic waves to better elucidate their involvement in immunological dysfunction and systemic alterations, also evaluating the possible impact of different pandemic waves.

Materials and methods

Patients and healthy donors

One hundred and thirty-three ($n = 133$) patients positive for SARS-CoV-2 (COV) were enrolled in an open-label study, promoted by the Departments of System Medicine and Experimental Medicine of the University of Rome, "Tor Vergata" in the Infectious Diseases Clinic of Policlinico "Tor Vergata" (PTV). The positivity to SARS-CoV-2 was asses by Allplex™ 2019-nCoV multiplex real-time polymerase chain reaction (PCR) assay, according to the manufacturer's instructions.

One hundred thirty-two ($n = 132$) individuals with post-acute sequelae of SARS-CoV-2 infection (PASC), hospitalized during the acute phase of COVID-19, were evaluated in the ambulatory of Infectious Diseases Clinic of Policlinico "Tor Vergata" (PTV) from three months after the acute phase of infection. Ethical approval for the collection and use of human samples was obtained from the Ethics Committee of 'Fondazione Tor Vergata', COrona VIrus Disease: Safety and efficacy of experimental treatment (COVID_SEET prot.7562/2020, 9 April 2020, experimental register 46.20). The COV and PASC groups were divided according to the COVID-19 waves found from March 2020 to February 2022, based on recruitment. In particular, 5 COVID-19 waves were identified, according to the literature: second wave (II) (September 2020-January 2021), third wave (III) (February 2021-June 2021), fourth wave (IV) (July 2021-September 2021), fifth wave (V)

(October 2021-February 2022) and sixth wave (VI) (from March 2022). The clinical and biochemical data from COV and PASC were collected and reported in Table 1 A and B. Blood samples from fifty-nine ($n = 59$) healthy donors (HD) were obtained from individuals attending the local blood transfusion center and referred to the Virology Unit for evaluation. To define baseline values for inflammatory index, data from 69 individuals who attended the local blood transfusion center were used. Donors were accepted to donate blood after signing informed consent and in accordance with current guidelines. Regarding SARS-CoV-2 infection, donors with a positive history of confirmed diagnosis of SARS-CoV-2 infection may be accepted for donation if at least 14 days have passed since complete resolution of symptoms, with the exception of ageusia/dysgeusia and anosmia that may persist for some time after recovery, or in the presence of a negative molecular or antigenic test performed according to public health timelines (Centro Nazionale Sangue Prot. n. 2022_0000069).

All donors have been matched for age and sex to the best possible extent with patients.

Analysis of CD169 and HLA-DR expression in blood cells by flow cytometry

Blood cells (80 μL) from COV, PASC and HD were vortexed for 5 min and incubated for 15 min in the dark at room temperature with 1.5 mL of VersaLyse Lysing Solution (Beckman Coulter, BC) to lyse red blood cells and select a total leukocyte population. Antibodies of interest were incubated for 15 min in the dark on ice. 10 μL of IOTest myeloid activation antibody cocktail composed of anti-CD169-phycoerythrin (PE) (clone 7–239), anti-CD64-Pacific Blue (PB) and HLA-DR (APC) (clone 22) (Beckman Coulter) was used. The stained cells were analysed via CytoFLEX (Beckman Coulter) and CytExpert 2.3 software (BC). CD169 expression was represented as the ratio of CD169 MFI in HLA-DR+ monocytes versus HLA-DR+ lymphocytes (RMFI) as described in a previous study (Minutolo et al., 2021). Furthermore, the percentage of positive monocytes for the HLA-DR and CD169 markers was also analyzed as reported in the gating strategy described in the **Supplementary Material 1**.

Statistical analysis

Statistical analysis of group-wise expression levels was performed using a nonparametric Mann Whitney test in the case of 2 independent samples and Bonferroni correction in the case of n-independent samples. Pairwise associations between continuous variables were tested using the Spearman correlation coefficient. Statistically significant comparisons were considered at $p < 0.05$. Data analyses were performed using SPSS statistical software (version 23.0 for Windows, USA).

Results

Demographics, biochemical and clinical values of COV patients and PASC individuals

This study included 133 patients with acute COVID-19 (COV, mean age ± standard deviation, 63 ± 13.81; 46 women and 87 men) and 132 individuals with post-acute sequelae of SARS-CoV-2 infection (PASC, mean age ± standard deviation, 58±14; 56 women and 76 men) (Table 1A). For PASC, the number of weeks after acute infection is also reported. Information regarding the severity of COVID-19 during the acute phase, treatments received, and time of vaccine administration were also reported in Table 1A. Regarding the severity score, 75.93 % of COVID-19 patients had moderate disease and 24.06 % had severe disease; among PASC subjects, referring to the acute phase of infection, 62.12 % had moderate disease and 31.81 % had severe disease. The samples of COVID-19 patients were analysed at the time of hospitalization, in concomitant with the starting of therapeutic treatment with antivirals, corticosteroids, and even monoclonal drugs. The type of treatment received was defined in detail: the 48.87 % of COVID-19 patients received antivirals, 30.08 % corticosteroids and 13.53 % monoclonal treatments. The 46.21 % of PASC individuals received antivirals, 46.97 % with corticosteroids and 21.97 % with monoclonal treatments. Regarding vaccination in COVID-19 patients, 55.63 % did not receive vaccination and 42.85 % were vaccinated before COVID-19. Among PASC individuals, 29.54 % did not receive vaccination, 19.69 % were vaccinated before and 45.45 % after COVID-19. Both COVID-19 patients and PASC individuals were categorized in different waves according to the acute phase period of COVID-19. In detail, the number of COVID-19 patients was: 47 in wave II (35.33 %), 14 in wave III (10.52 %), 0 in wave IV, 25 in wave V (18.79 %), and 25 in wave VI (18.79 %). The number of PASC individuals was: 19 in wave II (14.39%), 21 in wave III (15.90%), 13 in wave IV (9.84 %), 46 in wave V (34.84 %), and 21 in wave VI (15.90%).

Concerning the comorbidities was evaluated the following diseases: Diabetes, cardiovascular, obesity and tumor. Of the COVID-19 patients, 24.06 % had diabetes, 60.15 % had cardiovascular alterations, 16.57 % had obesity, and 21.05 % had cancer. Of the PASC individuals, 18.94 % had diabetes, 15.15 % had cardiovascular alterations, 19.70 % had obesity, and 6.82 % had cancer. The Charlson Comorbidity Index (CCI), used to classify comorbidities that could influence disease severity for both COV and PASC, was also reported. Finally, PASC individuals were also characterized according to the symptoms manifested after resolution of the infection (weeks after the acute infection IQR 16.00±36.25), showing different symptoms; each individual was evaluated for each symptom category. Of all PASC with respect to each 6 symptom group, 68.93 % showed systemic symptoms (asthenia, myalgia, fever, and joint pain); 49.24 % showed cardiorespiratory symptoms (dyspenaea, cough, chest pain, palpitation), 32.57 % showed cutaneous symptoms (rash, hair loss); 19.67 % showed gastrointestinal symptoms (diarrhoea, nausea, lack of appetite), 59.09 % showed neurological symptoms (cephalea, dysgeusia, dysosmia, hearing disturbances, paresthesias and tremors, and attention deficit), 39.39 % showed psychiatric symptoms (anxiety, depression, and emotional lability). Biochemical data analysis that showed statistically significant differences between the COV and PASC groups are signed with black asterisks. The hematological parameters such as hematocrit, platelets, eosinophils, and basophils were significantly lower in COV than in PASC, although they were in normal ranges. Neutrophils, on the other hand, were significantly higher in COV than PASC. Interestingly, red blood cells, lymphocytes, and hemoglobin were found to be outside the normal range in COV and significantly lower than in PASC (Table 1B). Analysis of coagulation parameters showed a significant difference in COV compared to PASC. In particular, in COV the fibrinogen and d-dimer were found to be out of normal ranges and significantly higher than in PASC, in which the value returned to normal. Taking into account clinical chemistry values, significant differences were found in COV with respect to PASC. Only in the COV, the parameters LDH (lactate dehydrogenase) and CRP (Reactive C Protein) were out of the normal range and significantly higher with respect to PASC. The Systemic Immune Inflammation index (SII, [neutrophil (N) x platelet (P) / lymphocyte (L)], 424.06 10^3/μL), Neutrophil to Lymphocyte ratio (NLR, 1.94), and Platelet to Lymphocyte Ratio (PLR, 127.30) were evaluated, and a score was generated to assess the systemic impact of the disease. Baseline values of these indices were calculated using 69 healthy donors (mean age ± standard deviation, 59.91 ± 3.80; 27 women and 41 men) matched by sex and age based on the COVID-19 patients and PASC individuals analyzed. As reported in Table 1B, the indices value calculated for COV and PASC were out of range and significantly higher in COV compared to PASC.

Evaluation of the CD169 and HLA-DR markers in monocytes from COV patients, PASC individuals, and HD

Based on our recent work (Minutolo et al., 2021), the ratio of CD169

Table 1
Clinical and biochemical data.

1A) Clinical features					
Samples		COV ($N = 133$)		PASC ($N = 132$)	
		Number	Percentage (%)	Number	Percentage (%)
SEX F/M		46/87	35/65	56/76	42/58
Age (mean±SD)		63±13.81		58±14	
Weeks after the-Acute Infection				Interquartile Range	
				(50)	(25–75)
				21.00	16.00- 36.25
Severity (Acute phase)					
	Moderate	101	75.93	82	62.12
	Severe	32	24.06	42	31.81
	N/A	0		10	7.57
Treatments					
Antiviral	Yes	66	48.87	61	46.21
	No	55	41.35	59	44.70
	N/A	12	9.02	12	9.09
Corticosteroids	Yes	40	30.08	62	46.97
	No	67	50.38	58	43.94
	N/A	25	18.80	12	9.09
Monoclonals	Yes	18	13.53	29	21.97
	No	114	85.71	91	68.94
	N/A	0	0	12	9.09
Vaccination					
	No	74	55.63	39	29.54
	Before	57	42.85	26	19.69
	After	0	0	60	45.45
	N/A	2	1.53	7	5.30
Waves					
	I	0	0	0	0
	II	47	35.33	19	14.39
	III	14	10.52	21	15.90
	IV	0	0	13	9.84
	V	25	18.79	46	34.84
	VI	25	18.79	21	15.90
Comorbidities					
Diabetes	Yes	32	24,06	25	18,94
	No	90	67,67	95	71,97
	N/A	11	8,27	13	9,09
Cardiovascular	Yes	80	60,15	20	15,15
	No	43	32,33	100	75,76
	N/A	10	7,52	13	9,09
Obesity	Yes	22	16,54	26	19,70
	No	101	75,94	94	71,21
	N/A	10	7,52	13	9,09
Tumor	Yes	28	21,05	9	6,82
	No	95	71,43	111	84,09
	N/A	10	7,52	13	9,09
Charlson Comorbidity Index		3.00***	IQR (2.00–5.50)	2.00	IQR (1.00–3.00)
		number	**%**	**number**	**%**
	0	8	6.4	**27**	21.8
	1	11	8.8	29	23.4
	2	**18**	**14.4**	**17**	13.7
	3	27	21.6	23	18.5
	4	19	15.2	7	5.6
	5	11	8.8	15	12.1
	6	**16**	12.8	**2**	1.6
	7	**5**	4	**0**	0
	8	**5**	4	**3**	2.4
	9	**1**	0.8	**0**	0
	10	**3**	2.4	**0**	0
	11	**0**	0	**0**	0
	12	**1**	0.8	**1**	0.8
PASC symptoms					
Systemic	No			30	22.72
	Yes			91	68.93
	N/A			11	8.33
Cardio-respiratory	No			56	42.42
	Yes			65	49.24
	N/A			11	8.33
Cutaneous	No			78	59.09
	Yes			43	32.57
	N/A			11	8.33
Gastrointestinal	No			96	72.72
	Yes			26	19.67
	N/A			10	7.57

(*continued on next page*)

Table 1 (*continued*)

1A) Clinical features Samples		COV ($N = 133$)		PASC ($N = 132$)	
		Number	Percentage (%)	Number	Percentage (%)
Neurological	No			43	32.57
	Yes			78	59.09
	N/A			11	8.33
Psychiatric	No			69	46.96
	Yes			52	39.39
	N/A			11	8.33

1B) Biochemistry Haematology	**Normal Range values**	Interquartile Range (50)	(25–75)	Interquartile Range (50)	(25–75)
Red blood cells	4.40–6.00 (10^6/μL)	**4.29*****	(3.66–4.68)	4.82	(4.48–5.13)
Hemoglobin	13–18 g/dL	**12.30** ***	(10.40–13.80)	13.90	(12.87–15.02)
Hematocrit	36–51 (%)	37.40***	(32.60–41.50)	41.55	(38.60–44.40)
Platelets	150–450 (10^3/μL)	215.00**	(154.00–291.00)	239.50	(210.75–290.00)
White blood cells	4.30–10.80 (10^5/uL)	6.30	(4.64–8.83)	6.63	(5.77–7.81)
Neutrophils	abs count 10^3/μL	4.33	(3.05–6.73)	3.85	(3.28–4.84)
	40–75 (%)	71.60***	(60.30–82.40)	60.15	(53.90–65.02)
Lymphocytes	abs count 10^3/μL	1.17***	(0.69–1.72)	1.96	(1.56–2.34)
	20–45 (%)	**19.40*****	(9.80–29.50)	29.80	(24.70–34.22)
Monocytes	abs count 10^3/μL	0.45	(0.34–0.69)	0.47	(0.40–0.56)
	3.4–11 (%)	7.20	(5.90–9.30)	7.10	(6.27–8.30)
Eosinophils	abs count 10^3/μL	0.01***	(0.00–0.06)	0.13	(0.06–0.21)
	0–7 (%)	0.20***	(0.00–0.80)	1.95	(1.10–3.50)
Basophils	abs count 10^3/μL	0.02***	(0.01–0.03)	0.04	(0.03–0.06)
	0–1.5 (%)	0.30***	(0.20–0.40)	0.60	(0.40–0.90)
Coagulation					
PT%	70–130 (%)	85.00***	(77.00–93.00)	102.00	(96.00–111.00)
PT-INR	0.80–1.20	1.12***	(1.04–1.20)	0.99	(0.94–1.03)
PT sec	*Sec*	13.30***	(12.40–14.20)	11.60	(1.25–12.20)
aPTT ratio	0.80–1.20	1.02	(0.92–1.11)	1.04	(0.98–1.12)
aPTT sec	25–38.50 (*sec*)	29.05*	(26.50–31.62)	30.15	(28.22–32.25)
Fibrinogen	200–400 (mg/dL)	**498.50*****	(379.00–608.00)	314.00	(265.00–382.00)
D-Dimer	0–500 (ng/mL)	**792.50*****	(478.75–1449.75)	304.50	(188.25–578.75)
Antitrombin	75–128 (%)	99.00	(89.00–112.00)	99.00	(88.00–106.00)
Clinical Chemistry					
Azotemia	18–55 (mg/dL)	42.00**	(28.75–66.50)	32.00	(26.00–40.50)
Potassium	3.50–5.10 (mEq/L)	4.10*	(3.80–4.55)	4.30	(4.00–4.40)
Albumin	3.20–4.60 (gr/dL)	3.49***	(3.08–4.08)	4.50	(4.15–4.70)
AST	5–34 (U/L)	29.00***	(21.00–45.00)	23.00	(19.00–27.75)
ALT	0–55 (U/L)	26.00	(13.00–40.75)	23.00	(17.00–28.00)
LDH	125–220 (U/L)	**278.00*****	(219.00–391.00)	197.00	(172.00–215.00)
Amylase	20–160 (U/L)	28.50	(42.00–79.25)	55.00	(49.00–70.00)
Lipase	<59 (U/L)	32.00	(24.00–50.00)	33.00	(24.50–40.00)
RCP	0–5 (mg/L)	**39.00*****	(23.75–89.25)	4.00	(2.50–5.00)
Inflammatory Indices					
SII	424.06 10^3/μL (320.16–538.50)	**864.94*****	(382.83–1684.26)	**486.94**	(338.00–665.65)
NLR	1.94 (1.31–2.41)	**3.62*****	(2.03–8.49)	**1.99**	(1.54–2.59)
PLR	127.30 (98.82–149.62)	**172.50*****	(124.82–315.93)	123.29	(97.89–162.07)

Bold for values out of normal range; Statistically significant differences between COV and PASC were indicated with black asterisks ***$p \leq 0.001$.
** $p \leq 0.01$.
* $p \leq 0.05$).

median fluorescence intensity (MFI) between HLA-DR+ activated monocytes and lymphocytes (CD169 RMFI) was evaluated in COVID-19 patients, PASC individuals, and healthy donors by flow cytometry (Fig. 1).

CD169 RMFI was found significantly elevated in COV compared to HD ($p < 0.001$) and to PASC ($p < 0.001$). No significant difference was observed in HD with respect to PASC ($p = 0.602$). In COV, the percentage of the CD169+ monocytes were significantly higher with respect to PASC ($p<0.001$) and HD ($p<0.001$), and, interestingly, a significant difference between PASC and HD was observed ($p <0.001$). The HLA-DR+ monocyte analysis revealed a significantly lower percentage in COV ($p < 0.001$) and PASC ($p< 0.001$) with respect to HD, while the percentage of HLA-DR+ monocytes of COV and PASC was comparable ($p = 0.657$). Furthermore, the percentage of HLA-DR+CD169+ monocytes was evaluated. The percentage of HLA-DR+CD169+ monocytes was significantly higher in COV than in HD ($p < 0.001$) and PASC ($p < 0.001$). Notably, PASC individuals maintained a significantly higher percentage of HLA-DR+CD169+ monocytes with respect to HD ($p = 0.020$).

Evaluation of inflammatory indices (SII, NLR, PLR) in COV and PASC

Taking into account the important systemic alteration found in both COV and PASC, three inflammatory indices reflecting inflammation and immune system alteration were calculated (Ghobadi et al., 2022). The systemic immune inflammation index (SII), which considers the number of neutrophils, platelets, and lymphocytes, was analyzed and represented as box plots in Fig. 2. The SII value of the COV group was significantly higher with respect to PASC ($p<0.001$). Interestingly, both COV ($p<0.001$) and PASC ($p = 0.021$) showed a higher value than HD, reported as a reference value.

The neutrophil/lymphocyte ratio (NLR) was also evaluated, demonstrating higher values in COV with respect to PASC. Taking into account the normal value calculated in the HD group, only COV showed

Table 2
Impact of different COVID-19 waves on CD169 RMFI, CD169, and HLA-DR expression on COV monocytes.

	COV			
	RMFI CD169	CD169+ monocytes(%)	HLA-DR+ monocytes(%)	HLA-DR+ CD169+ monocytes(%)
II vs HD	**0.000**	**0.000**	**0.000**	**0.000**
III vs HD	**0.000**	**0.000**	**0.084**	**0.000**
V vs HD	**0.000**	**0.000**	**0.000**	**0.000**
VI vs HD	**0.000**	**0.000**	**0.000**	**0.000**
II vs III	0.763	0.430	0.643	0.471
II vs V	0.073	0.109	**0.003**	0.158
II vs VI	**0.001**	**0.035**	**0.024**	**0.031**
III vs V	0.381	0.693	0.079	0.725
III vs VI	0.152	0.583	0.138	0.41
V vs VI	0.512	0.855	0.058	0.657
HD vs PASC	0.602	**0.000**	**0.000**	**0.000**
II vs PASC	**0.000**	**0.000**	0.131	**0.000**
III vs PASC	**0.000**	**0.000**	0.191	**0.000**
V vs PASC	**0.000**	**0.000**	**0.017**	**0.000**
VI vs PASC	**0.000**	**0.000**	0.151	**0.000**

The Mann Whitney test was used to compare the analyzed groups.
Bold for significant values; p value ≤ 0.05.

Table 3
The impact of different COVID-19 waves to CD169 RMFI, CD169 and HLA-DR expression on monocytes of PASC individuals.

	PASC			
	RMFI CD169	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+ CD169+ monocytes (%)
II vs HD	0.438	0.889	**0.000**	**0.000**
III vs HD	0.410	**0.005**	**0.000**	**0.000**
IV vs HD	0.541	**0.007**	**0.001**	**0.000**
V vs HD	0.482	**0.000**	**0.000**	**0.000**
VI vs HD	0.557	0.955	**0.000**	**0.000**
II vs III	0.308	**0.053**	0.150	0.060
II vs IV	0.866	**0.042**	0.420	0.057
II vs V	0.356	**0.008**	**0.008**	**0.009**
II vs VI	0.901	0.857	0.901	0.923
III vs IV	0.703	0.456	0.127	0.775
III vs V	0.703	0.456	0.127	0.775
III vs VI	0.290	0.082	0.062	0.141
IV vs V	0.585	0.952	0.005	0.686
IV vs VI	0.710	0.083	1.000	0.137
V vs VI	0.350	**0.013**	**0.000**	**0.031**

The Mann Whitney test was used to compare the analyzed groups. **Bold** for significant values; p value ≤ 0.05.

a significant difference ($p<0.001$). Inversely, PASC did not show differences from normal values ($p = 0.157$).

Also, for the platelet/lymphocyte ratio (PLR) a higher value in COV with respect to PASC ($p<0.001$) and with respect to the normal range were observed ($p<0.001$).

Evaluation of RMFI and expression of CD169 and HLA-DR markers in COV patients and PASC individuals in association with COVID-19 waves

The impact of COVID-19 waves on CD169 RMFI and the expression of HLA-DR+ and CD169+ on monocytes in the total COV and PASC were then studied. The COV and PASC were stratified into five categories according to the acute phase of COVID-19 (Fig. 3 and Fig. 4). Specifically, considering the COV, 47 were enrolled in wave II, 14 in wave III, 25 in wave V, and 47 in wave IV. In each different wave, the COV showed a significantly higher CD169 RMFI, percentage of CD169+ and CD169+HLA-DR+ monocytes compared with HD and PASC. Furthermore, for all the parameters but CD169 RMFI there was a significant difference between HD and PASC.

Taking into account the COV, wave II showed significantly higher CD169 RMFI ($p = 0.001$), percentage of CD169+ ($p = 0.035$), HLA-DR+ ($p = 0.024$) and HLA-DR+CD169+($p = 0.031$) monocytes than the VI wave. Compared to wave II, COV infected during wave V presented a significantly low percentage of HLA-DR+ monocytes ($p = 0.003$).

Also, PASC individuals were classified into categories according to the acute phase period of COVID-19. In detail, the number of individuals was 19 in wave II, 22 in wave III, 13 in wave IV, 46 in wave V, and 22 in wave VI (Fig. 4). All the parameters referred to the sampling time that occurred in the post-acute infection period, with no evidence of SARS-CoV-2 infection persistence. There were no significant differences in CD169 RMFI in PASC compared to HD and among the different waves, confirming CD169 RMFI as a marker of active viral infection. PASC from waves II to VI showed a lower percentage of HLA-DR+ monocytes and higher percentages of CD169+HLA-DR+ monocytes with respect to HD. The percentage of CD169+ monocytes was significantly lower in PASC individuals of which the acute phase of COVID-19 corresponded to wave II when compared with those in IV ($p = 0.042$) and V ($p = 0.008$). Interestingly, PASC of wave V showed the highest expression of CD169, HLA-DR+, CD169+HLA-DR+ monocytes both compared with wave II ($p = 0.008$; $p = 0.008$; $p = 0.009$) and VI ($p = 0.013$; $p<0.001$; $p = 0.031$).

Analysis of inflammatory indices (SII, NLR, PLR) in COV and PASC groups on different COVID-19 waves

To assess the contribution of different COVID-19 waves to inflammatory indices, COV patients (Fig. 5) and PASC individuals (Fig. 6) were stratified according to time of infection as reported in materials and methods. Overall, SII (systemic immune inflammation index reported as the number of neutrophils* platelets/lymphocytes), NLR (neutrophil/lymphocyte ratio) and PLR (platelet/lymphocyte ratio) values were significantly higher in COV patients (regardless of COVID-19 waves) than the range of healthy donors, confirming a systemic alteration due to COVID-19 in all the waves. In particular, patients infected during the third wave showed the highest levels of inflammatory indices, and no significant differences were observed between the third and fifth waves (SII $p = 0.102$, NLR $p = 0.180$, PLR $p = 0.91$), suggesting that these two waves may have had the most important contribution to systemic alteration due to COVID-19 (Table 4). Although the SII, NLR, and PLR values in PASC were statistically lower than those of COV stratified by COVID-19 waves, in PASC individuals the SII were significantly higher compared to baseline values of healthy donors ($p = 0.021$), as already shown in Fig. 2. Interestingly, no statistically significant differences were observed between PASC and COV-infected during wave II ($p = 0.262$), highlighting a similar systemic alteration between these 2 groups.

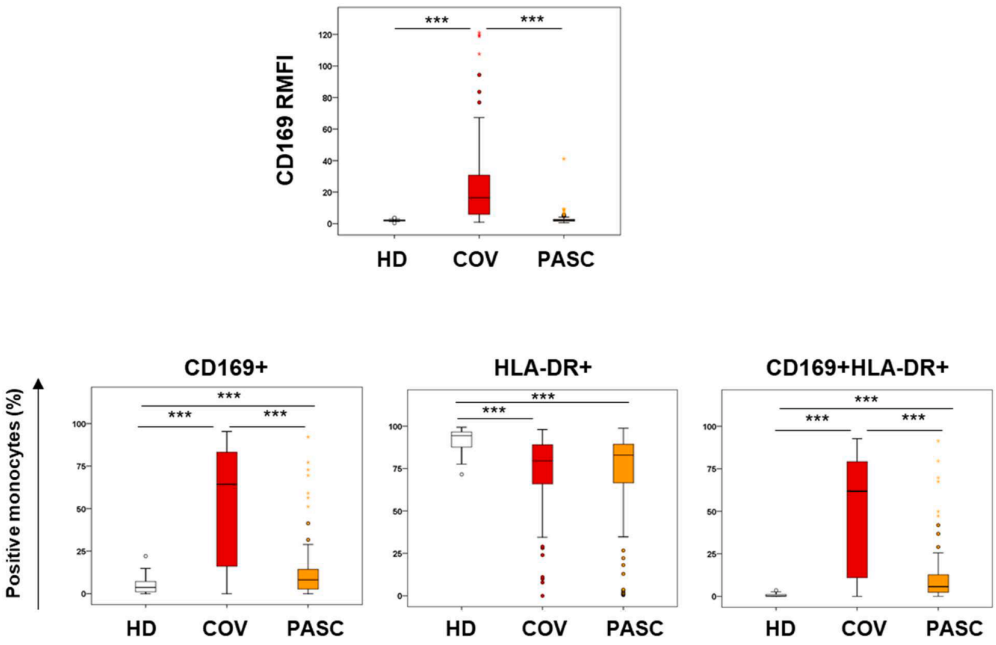

Fig. 1. Analysis of CD169 RMFI and the percentage of HLA-DR+ and CD169+ monocytes cells in COV, PASC, and HD. Representation of CD169 RMFI, defined as the ratio of the mean fluorescence intensity (MFI) of CD169 in HLA-DR+ monocytes and lymphocytes, evaluated in COV ($n = 133$, red box), PASC ($n = 132$, orange box) and HD ($n = 59$, white box). For the analysis of the percentage of CD169+, HLA-DR+, and HLA-DR+CD169+ monocytes, the percentage of positive cells was determined as shown in the gating strategy **in supplementary material 1**. The Mann Whitney test was used to compare groups (***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$).

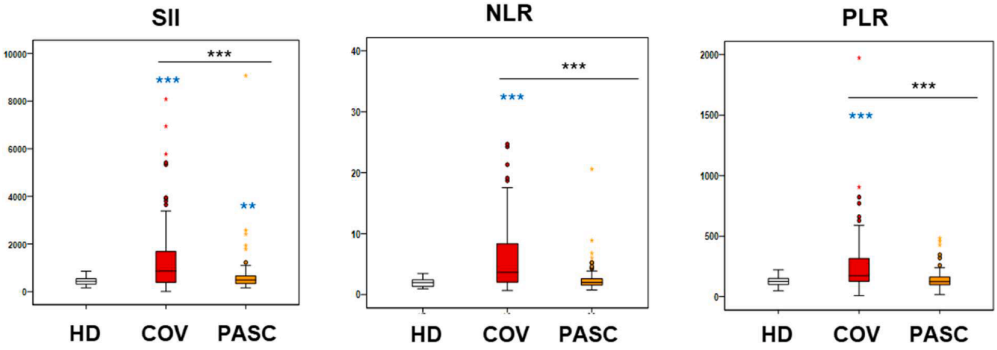

Fig. 2. Analysis of SII, NLR, and PLR in COV and PASC. Normal range values from HD ($n = 69$) were calculated (SII: 424.06; NLR: 1.94; PLR: 127.30) and reported in white box plots. The Mann-Whitney test was used to compare the analyzed groups (***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; blue asterisk defines the differences with respect to HD). SII (systemic immune inflammation index reported as the number of neutrophils* platelets/lymphocytes), NLR (neutrophil/lymphocyte ratio), PLR (platelet/lymphocyte ratio).

Stratifying the PASC individuals according to the wave of their acute phase of COVID-19, some statistically significant differences were found in inflammatory indices values (Fig. 6). Specifically, higher SII and NLR were observed in PASC individuals in wave VI compared with the baseline values of healthy donors (SII $p = 0.029$, NLR $p = 0.002$), and PASC individuals of wave V showed elevated levels of SII compared to HD ($p = 0.009$). Moreover, PASC individuals of wave V showed higher levels of SII with respect to wave III ($p = 0.035$). Regarding the NLR values, the PASC individuals of wave VI showed higher values than wave II ($p = 0.048$), wave III ($p < 0.001$), and wave V ($p = 0.020$). No statistically significant differences were observed for PLR values between PASC individuals divided by wave.

Correlation analysis of CD169 and HLA-DR with hematological, coagulation, biochemical parameters and inflammatory indices in COVID-19 and PASC

The association of CD169 and HLA-DR with clinical markers and inflammatory indices in COV patients and PASC individuals was evaluated (Tables 5-7). Regarding the hematologic parameter (Table 5), the CD169 RMFI, CD169+ monocytes, and the HLA-DR+CD169+ monocytes in COV patients were positively correlated with Hemoglobin (Rho=0.192, $p = 0.029$; Rho=0.266, $p = 0.020$; Rho=0.235, $p = 0.070$ respectively), and Hematocrit (Rho=0.175, $p = 0.047$; Rho=0.245, $p = 0.005$; Rho=0.215, $p = 0.013$ respectively) and negatively with white blood cell counts (Rho=−0.313, $p < 0.001$; Rho=−0.201, $p = 0.021$; Rho=−0.203, $p = 0.020$), as well as with the absolute count of the white blood cell subpopulations (see Table 6). Specifically, in COV the CD169

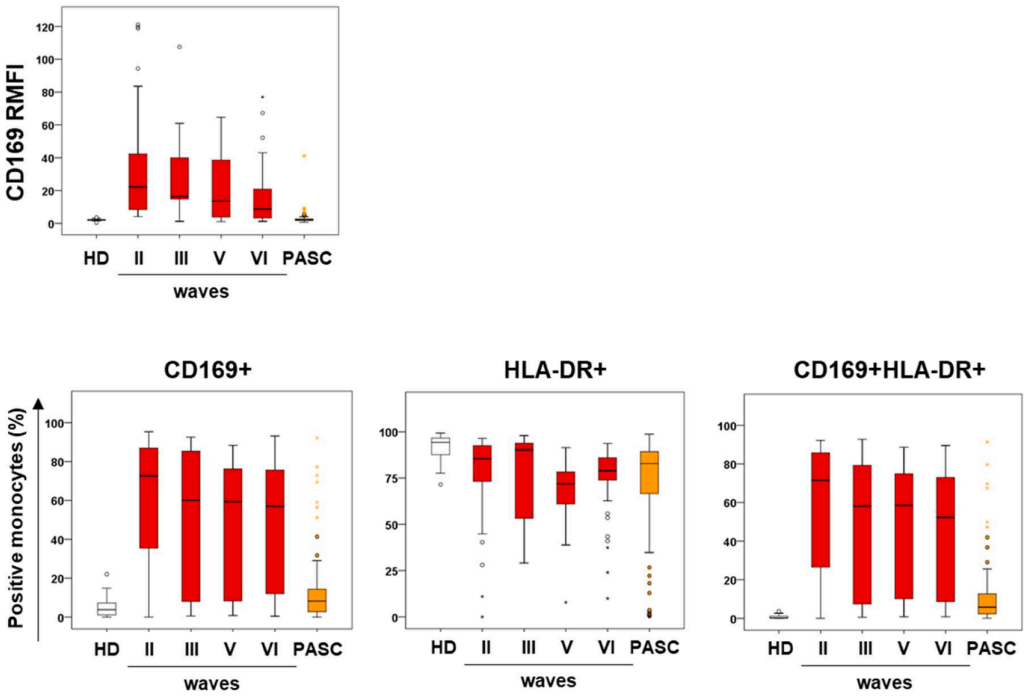

Fig. 3. Impact of different COVID-19 waves on CD169 RMFI, CD169 and HLA-DR expression on COV monocytes. COV ($n = 133$) were divided according to the acute phase of COVID-19: second wave (II) (September 2020-January 2020), third wave (III) (February 2021-June 2021), fifth wave (V) (October 2021-February 2022), and sixth wave (VI) (from March 2022). CD169 RMFI, percentage of CD169+, HLA-DR+, and CD169+HLA-DR+ monocytes were analyzed. The Mann-Whitney test was used to compare the analyzed groups and the statistical results are reported in Table 2.

RMFI inversely correlated with platelets (Rho = −0.211, $p = 0.016$), and this trend was confirmed also in PASC individuals (Rho=−0.200, $p = 0.028$). CD169 RMFI was also inversely correlated with eosinophil and basophil count in COV, while in PASC individuals a direct and significant correlation was observed between CD169+, HLADR+, HLA-DR+CD169+ monocytes and eosinophil and basophil absolute count and percentage. Furthermore, in PASC individuals, a direct correlation of CD169+ and HLA-DR+CD169+ monocytes with the percentage of Lymphocytes was found (Rho=0.237, $p = 0.09$; Rho=0.234, $p = 0.010$ respectively).

Regarding coagulation markers (Table 6), in COV patients a direct and significant correlation was observed between CD169 RMFI and Ptper, Antithrombin and Fibrinogen (Rho=0.209, $p = 0.029$; Rho=0.198, $p = 0.047$; Rho=0.199, $p = 0.039$ respectively). These correlations were lost in PASC but a direct and significant correlation was observed in CD169+ and CD169+ monocytes with respect to APTTsec (Rho=0.249, $p = 0.010$; Rho=0.212, $p = 0.028$ respectively).

In COV patients, a direct correlation was observed between CD169+, HLA-DR+ and HLA-DR+CD169+ monocytes with respect to albumin (Rho=0.368, $p = 0.004$; Rho=0.277, $p = 0.032$; Rho=0.354, $p = 0.006$ respectively), while in PASC an inverse correlation between CD169 RMFI and albumin (Rho = 0.−537, $p = 0.039$) and a direct association with AST (Rho=0.212, $p = 0.018$) were found. Moreover, in COV, the percentage of HLA-DR+ monocytes was inversely correlated with the LDH and CRP values (Rho=0.255, $p = 0.005$; Rho=−0.571, $p = 0.013$).

Finally, with respect to comorbidities and immune system indices (Table 7), no significant correlation was observed in COV. Interestingly, in PASC individuals, a significant direct correlation between the Charlson index and CD169 RMFI, and an inverse association with SII (Rho=0.201, $p = 0.028$; Rho=−0.259, $p = 0.004$ respectively) were found. The CD169+ and HLA-DR+CD169+ populations were also inversely correlated with the NLR index (Rho=−0.274, $p = 0.002$; Rho=−0.260, $p = 0.004$ respectively).

Discussion

In this study we demonstrated the alteration of CD169 and HLA-DR markers in monocytes in COVID-19 patients and in individuals with post-acute sequelae of SARS-CoV-2 infection. Furthermore, we observed high values of systemic inflammatory indices such as SII, NLR, and PLR, in patients with COVID-19 and PASC individuals, highlighting the persistence of systemic alteration after the resolution of the infection. In this scenario, we studied the contribution of different COVID-19 waves to the expression of CD169 and HLA-DR and systemic inflammation indices to evaluate the impact of the complexity of COVID-19 and the sequelae of post-infection.

We confirmed in a wide number of COVID-19 patients enrolled during different pandemic waves that CD169 RMFI is a reliable and robust marker of active SARS-CoV-2 infection, reinforcing the data reported in our and other research's previous works (Bourgoin et al., 2021; Doehn et al., 2021; Minutolo et al., 2021). Moreover, in PASC individuals no significant CD169 RMFI was found compared to HD, demonstrating its specificity during viral infections.

The COVID-19 patients, as previously reported, showed several biochemical parameters outside the normal range, such as a number of red blood cells (Bouchla et al., 2022), hemoglobin, percentages of lymphocytes, fibrinogen, and d-Dimer, LDH, RCP, and together with altered system inflammatory indices (SII, NLR, PLR) (Kosidło et al., 2023). Interestingly, in our cohort, the CD169 RMFI was found directly correlated with fibrinogen and hemoglobin, which are recognized as important indicators of poor prognosis (Lee et al., 2021; Leentjens et al., 2021). Furthermore, the high levels of CD169 RMFI were found also inversely correlated with eosinophil and basophil count in the acute phase of infection. Recently evidence indicates eosinophils as potential markers of severity prediction of disease in respiratory virus infections and in COVID-19 (Macchia et al., 2023). Specifically, eosinopenia was demonstrated as an indicator of severity, probably associated to immune hyperactivation and cytokine storm characterizing severe and critical

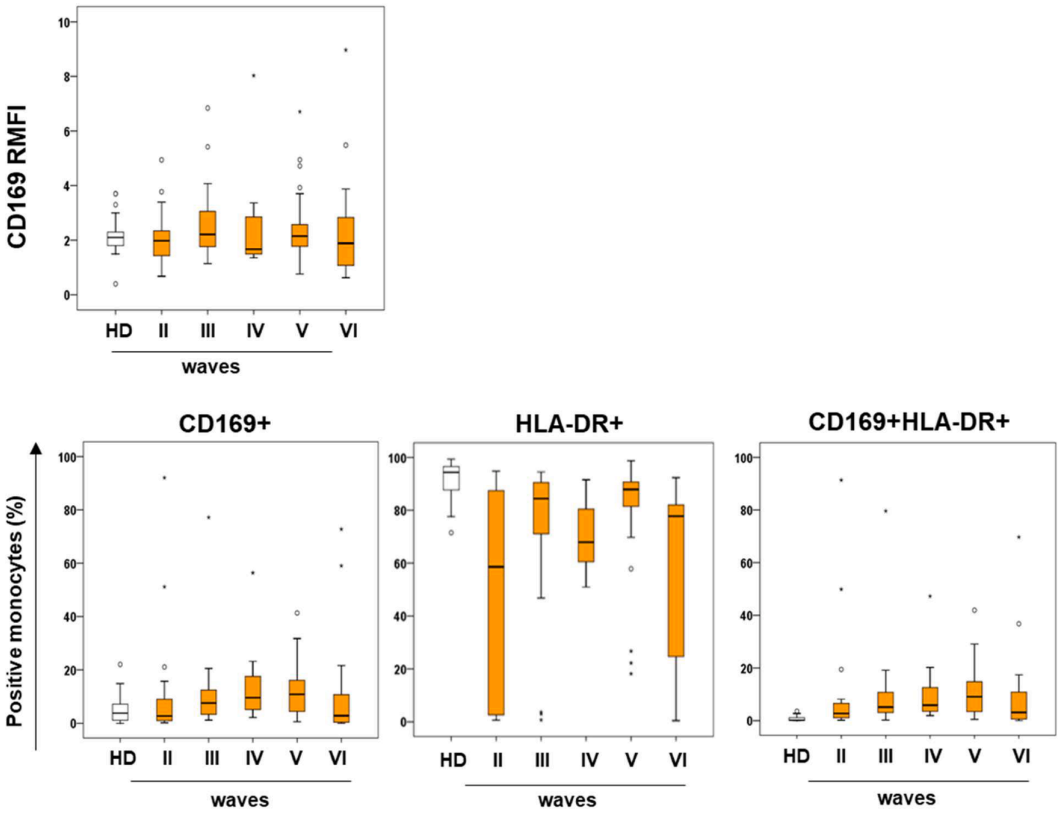

Fig. 4. The impact of different COVID-19 waves on CD169 RMFI, CD169 and HLA-DR expression on monocytes of PASC individuals. PASC ($n = 122$) were divided according to the COVID-19 waves found from March 2020 to February 2022, based on recruitment: second wave (II) (September 2020-January 2021), third wave (III) (February 2021-June 2021), fourth wave (IV) (July 2021-September 2021), fifth wave (V) (October 2021-February 2022) and sixth wave (VI) (from March 2022). The CD169 RMFI, percentage of CD169+, HLA-DR+ and CD169+HLA-DR+ monocytes were analyzed. Mann Whitney test was used to compare groups and the statistical results were reported in Table 3.

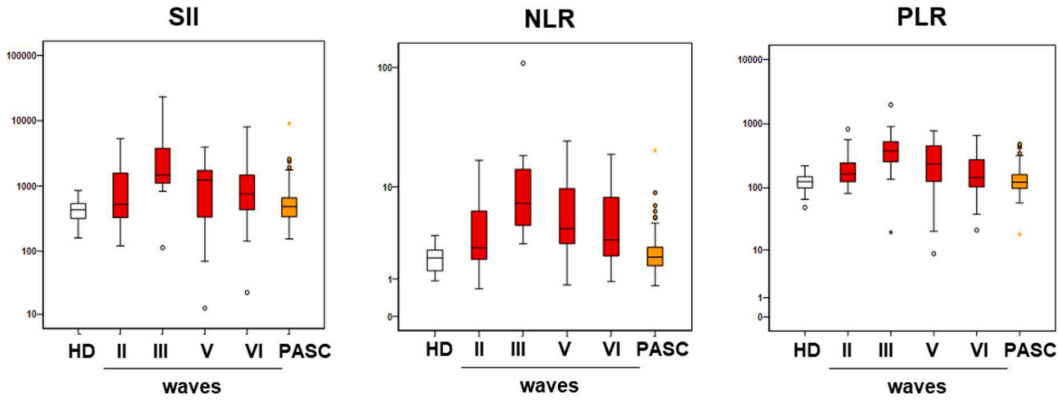

Fig. 5. Analysis of inflammatory indices (SII, NLR, PLR) in COV patients upon different COVID-19 waves. COV ($n = 133$) were divided according to the period of acute phase of COVID-19: second wave (II) (September 2020-January 2020), third wave (III) (February 2021-June 2021), fifth wave (V) (October 2021-February 2022) and sixth wave (VI) (from March 2022). SII (systemic immune inflammation index reported as the number of neutrophils* platelets/lymphocytes), NLR (neutrophil/lymphocyte ratio) and PLR (platelet/lymphocyte ratio). Mann Whitney test was used to compare the groups analyzed and the statistical results were reported in Table 4.

COVID-19 patients (Cauchois et al., 2022; Koc et al., 2022).

The CD169 RMFI in PASC was found to be very low but showed correlations with some biochemical parameters such as negatively with albumin and SII, and positively with the Charlson Comorbidity Index and the AST. It is worth mentioning that the PASC individuals, especially from more recent pandemic waves (V and VI), showed elevated systemic immune inflammation index and NLR, confirming the persistence of systemic alteration and the impaired immune response as characteristic of these individuals. Indeed, together with the presence of heterogeneous symptoms (neurological, cardiorespiratory, psychiatric, systemic, gastrointestinal) (Chevinsky et al., 2021; CDC, 2022), PASC individuals often show elevated levels of pro-inflammatory cytokines such as IL-1β,

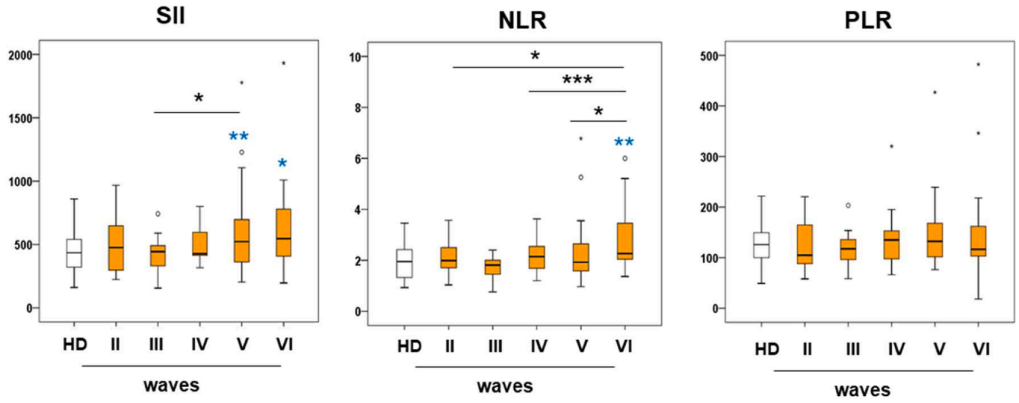

Fig. 6. Analysis of inflammatory indices (SII, NLR, PLR) in PASC individuals upon different COVID-19 waves. PASC ($n = 122$) were divided according to the COVID-19 waves found from March 2020 to February 2022, based on recruitment: second wave (II) (September 2020-January 2021), third wave (III) (February 2021-June 2021), fourth wave (IV) (July 2021-September 2021), fifth wave (V) (October 2021-February 2022) and sixth wave (VI) (from March 2022). SII (systemic immune inflammation index reported as the number of neutrophils *x* platelets/lymphocytes), NLR (neutrophil/lymphocyte ratio) and PLR (platelet/lymphocyte ratio). Mann Whitney test was used to compare groups (***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; blue asterisks define statistically significant differences with respect to HD).

Table 4
Analysis of inflammatory indices (SII. NLR. PLR) in COV patients upon different COVID-19 waves. .

	COV		
	SII	NLR	PLR
II vs HD	**0.032**	**0.000**	**0.000**
III vs HD	**0.000**	**0.000**	**0.000**
V vs HD	**0.001**	**0.000**	**0.001**
VI vs HD	**0.000**	**0.000**	**0.022**
II vs III	**0.006**	**0.001**	**0.002**
II vs V	0.352	0.056	0.199
II vs VI	0.310	0.325	0.357
III vs V	0.102	0.180	0.191
III vs VI	**0.007**	**0.005**	**0.001**
V vs VI	0.521	0.146	0.122
HD vs PASC	**0.021**	0.157	0.63
II vs PASC	0.262	**0.002**	**0.000**
III vs PASC	**0.000**	**0.000**	**0.000**
V vs PASC	**0.004**	**0.000**	**0.001**
VI vs PASC	**0.002**	**0.000**	**0.036**

The Mann Whitney test was used to compare the analyzed groups.
Bold for significant values; p-value ≤ 0.05.

IL-6, TNFα (Holms, 2022) and IL-17, IL-2, IL-4, IL-10 (Queiroz et al., 2022), probably determining their persistent inflammation (O'Laughlin et al., 2022). Hyperinflammation, defined as cytokine storm and exhaustion of uncontrolled activation of the immune system represents a characteristic of COVID-19, which, reflected at the systemic level, leads to a severe imbalance. The evidence in PASC individuals is a protracted state of this altered condition that manifests itself not only with evident symptoms but also with the establishment of chronic inflammation (Afrin et al., 2020).

Myeloid cells are responsible for the pathophysiology of COVID-19 by acting as potential cytokine producers leading to hyper-inflammation (Qin et al., 2021). Several works have demonstrated the high presence of CD169+ monocytes in the early stages of COVID-19 (Chevrier et al., 2020; Martineza et al., 2020) promoting the progression of inflammation (Ait Belkacem et al., 2021). In agreement, our results showed a higher percentage of CD169+ monocytes in COVID-19 patients compared to healthy donors. Notably, for the first time we demonstrated in PASC individuals a higher percentage of CD169+ monocytes compared to HD, suggesting their involvement in the maintenance of hyperinflammation also during post-infection sequelae.

In addition to hyperinflammation and cellular exhaustion (Mazzoni et al., 2021), another important feature of COVID-19 is immunosuppression (Metha et al., 2020). Moreover, it is well known how reduced expression of the HLA-DR (class II human leukocyte antigen) in monocytes plays a key role in this dysfunctional immune response, especially in critical COVID-19 patients (Spinetti et al., 2020.; Hasan et al., 2022). In the present study, we demonstrated a lower percentage of HLA-DR+ monocytes in COVID-19 patients compared to healthy donors, and inversely correlating with poor prognostic parameters of LDH and RCP. In particular, we found for the first time that in PASC individuals the percentage of HLA-DR+ monocytes was lower than in healthy donors and comparable with COVID-19 patients. This result supports the failure to recover the normal immune response in PASC individuals after the acute phase of COVID-19. Indeed, the decrease in HLA-DR expression on monocytes represents a specific hallmark of immune imbalance in patients with systemic inflammatory response syndrome and immunodepression (Kim et al., 2010). HLA-DR antigen polymorphisms are known to influence the immune response and disease outcome, exerting an effect on the presence of symptoms in SARS-CoV-2 infection. This mechanism could underlie persistent immune deregulation and sequelae found in PASC individuals (Astbury et al., 2022). Furthermore, we observed that the coexpression of HLA-DR and CD169 on monocytes was significantly higher in COVID-19 patients with respect to HD and PASC. Despite the low percentage of HLA-DR+CD169+ monocytes in PASC, although not apparently infected, this population should be considered since found significantly higher with respect to HD. Taking into account the role of this cell population during infection, our results could suggest the presence of protein and residual material (RNA) of the virus present in the bloodstream or in extracellular vesicles (Barberis et al., 2021; Craddock et al., 2023). Intriguingly, the persistence of CD169+ HLA-DR+ monocytes in PASC individuals could be caused by a possible SARS-CoV-2 reservoir or a persistent immune dysfunction (Proal et al., 2023).

Indeed, CD169+ monocytes expressing high levels of co-stimulatory molecules and HLA have been shown to be involved in an increased activation state, particularly towards CD8+ *T* cell stimulatory capacity (Affandi et al., 2021). In agreement, we have already demonstrated in a previous work that CD169 RMFI is also correlated with senescence and exhaustion of the CD8 T cell subset (Minutolo et al., 2021), suggesting the persistence of circulating CD169+ monocytes in PASC in sustaining the CD8 T cell exhaustion due to a persistent co-stimulation.

Considering treatments, only a small percentage of COVID-19 patients had received home therapies before hospitalization (9 %), and a part received drugs such as antivirals, corticosteroids, and also

Table 5
Spearman coefficient correlation and P value of CD169 and HLA-DR with Hematological parameters associated with disease severity in Acute COVID-19 and their association with the PASC phenotype.

HEMATOLOGY									
		COV				PASC			
		CD169 RMFI	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+CD169+ monocytes (%)	CD169 RMFI	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+CD169+ monocytes (%)
Red blood cells	Rho	0.116	**0.175***	0.125	0.157	-0.090	0.038	-0.010	-0.025
	p	0.191	**0.045**	0.155	0.072	0.325	0.677	0.913	0.787
Hemoglobin	Rho	**0.192***	**0.266****	0.146	**0.235****	-0.105	0.029	0.084	-0.033
	p	**0.029**	**0.002**	0.095	**0.007**	0.251	0.755	0.360	0.719
Hematocrit	Rho	**0.175***	**0.245****	0.130	**0.215***	-0.068	0.083	0.145	0.030
	p	**0.047**	**0.005**	0.139	**0.013**	0.457	0.362	0.110	0.739
Platelets	Rho	**-0.211***	-0.064	0.024	-0.073	**-0.200***	0.050	0.127	0.009
	p	**0.016**	0.466	0.785	0.405	**0.028**	0.583	0.164	0.925
White blood cells	Rho	**-0.313****	**-0.201***	-0.019	**-0.203***	-0.108	**-0.184***	0.050	-0.151
	p	**0.000**	**0.021**	0.830	**0.020**	0.239	**0.042**	0.585	0.096
Neutrophils	Rho	**-0.272****	**-0.199***	-0.013	**-0.189***	**-0.184***	**-0.274****	-0.018	**-0.242****
	p	**0.002**	**0.023**	0.880	**0.031**	**0.043**	**0.002**	0.840	**0.007**
Monocytes	Rho	**-0.447****	-0.152	0.063	**-0.179***	0.061	-0.048	**0.231***	-0.010
	p	**0.000**	0.084	0.475	**0.040**	0.504	0.596	**0.010**	0.911
Eosinophils	Rho	**-0.291****	-0.027	0.104	-0.046	0.024	**0.266****	**0.183***	**0.308****
	p	**0.001**	0.759	0.235	0.605	0.794	**0.003**	**0.043**	**0.001**
Basophils	Rho	**-0.252****	-0.030	0.027	-0.047	0.086	**0.187***	0.125	**0.197***
	p	**0.004**	0.736	0.761	0.591	0.348	**0.039**	0.169	**0.029**
Neutrophils (%)	Rho	-0.039	-0.087	-0.029	-0.054	**-0.188***	**-0.307****	-0.171	**-0.302****
	p	0.664	0.321	0.746	0.538	**0.039**	**0.001**	0.060	**0.001**
Lymphocytes (%)	Rho	0.140	0.149	0.036	0.120	0.107	**0.237****	0.079	**0.234****
	p	0.114	0.089	0.681	0.173	0.243	**0.009**	0.386	**0.010**
Monocytes (%)	Rho	-0.152	0.029	0.081	-0.002	**0.183***	0.156	**0.232***	0.187*
	p	0.085	0.739	0.357	0.984	**0.045**	0.086	**0.010**	0.039
Eosinophils (%)	Rho	**-0.221***	0.051	0.139	0.028	0.067	**0.321****	0.175	**0.358****
	p	**0.012**	0.562	0.113	0.749	0.467	**0.000**	0.054	**0.000**
Basophils (%)	Rho	-0.135	0.136	0.088	0.110	0.141	**0.256****	0.095	**0.264****
	p	0.128	0.121	0.320	0.210	0.123	**0.004**	0.296	**0.003**

Bold for significantly values (p value ≤ 0.05)

monoclonal drugs at the time of hospitalization. Statistical analysis showed that there were no significant differences in the expression of the HLA-DR+, CD169+, and HLA-DR+CD169+ monocytes considering variable treatment with antivirals and corticosteroids. While monoclonal administration, although used in a small population (18/133), resulted in a significant lower percentage of CD169 monocytes, even if it still higher than in healthy donors. This could depend on the type of drug that certainly has a more specific effect on immunologic modulations, and it will certainly be interesting to observe in larger case series the effects of this therapy. We also observed that COVID-19 patients who received vaccination prior to infection (42.85 %), showed lower percentage of CD169+ and HLA-DR+ monocytes compared to no vaccinated COVID-19 patients. Interestingly, we have already demonstrated that CD169 is an early marker of SARS-CoV-2 infection, also induced after in vitro stimulation of PBMCs with SPIKE protein, which correlated with the severity of the disease (Minutolo et al., 2022). The low values found in vaccinated acute patients is in agreement with the well-known effect of vaccination in reducing the severity of infection, but more studies are needed to delineate the role of this marker in COVID-19.

Several pieces of evidence highlighted how the absence of immediate care and treatment, rapid diagnosis by fast antigen testing, variants with higher transmissibility, use of masks and administration of vaccines distinguished the different waves (El-Shabasy et al., 2022). Hence, we also pointed attention to analyzing COVID-19 patients and PASC individuals stratified by pandemic waves at the time of infection. The values of CD169+ and HLA-DR+CD169+ monocyte, and the SII, NLR, and PLR values, were higher in patients with COVID-19 than healthy donors regardless of waves, underlining the critical role of systemic alteration due to SARS-CoV-2 as a critical feature of COVID-19 independently of the period of infection. High RMFI of CD169 was confirmed as a marker of early viral infection and found to be elevated in COVID-19 patients from all pandemic waves analyzed. Interestingly there was a decrease in values from wave II to wave VI, together with a reduction on the percentage of CD169+, HLA-DR+, and CD169+HLA-DR+ monocytes. These results could depend on several factors, such as different variants of SARS-CoV-2 prevalent at that time, the impact of different devices and strategies to counteract the spread of the virus (molecular swabs, masks, isolation), and vaccine administration that also changed the incidence of respiratory viruses in the pandemic era (Costanza et al., 2022).

Table 6
Spearman correlation coefficient and P value of CD169 and HLA-DR with Clinical Chemistry parameters associated with disease severity in Acute COVID-19 and their association with the PASC phenotype.

CLINICAL CHEMISTRY

		COV				PASC			
		CD169 RMFI	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+CD169+ monocytes (%)	CD169 RMFI	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+CD169+ monocytes (%)
Ptper	Rho	**0.209***	0.117	0.168	0.131	0.035	-0.054	-0.091	-0.066
	p	**0.029**	0.221	0.077	0.169	0.718	0.580	0.350	0.496
aPTT ratio	Rho	0.007	-0.106	**-0.225***	-0.107	0.134	0.171	0.123	0.145
	p	0.941	0.269	**0.018**	0.264	0.171	0.078	0.209	0.137
aPTT sec	Rho	-0.027	-0.165	**-0.244***	-0.161	0.183	**0.249****	0.137	**0.212***
	p	0.778	0.085	**0.010**	0.092	0.061	**0.010**	0.161	**0.028**
Antitrombin	Rho	**0.198***	0.047	**0.219***	0.056	0.025	-0.183	-0.118	-0.146
	p	**0.047**	0.640	**0.026**	0.576	0.915	0.428	0.609	0.527
Fibrinogen	Rho	**0.199***	0.032	-0.171	0.017	0.006	-0.055	-0.022	-0.065
	p	**0.039**	0.742	0.073	0.859	0.950	0.570	0.817	0.500
Albumin	Rho	0.106	**0.368****	**0.277***	**0.354****	**-0.537***	-0.488	-0.483	-0.452
	p	0.425	**0.004**	**0.032**	**0.006**	**0.039**	0.065	0.068	0.090
AST	Rho	0.161	0.069	-0.056	0.057	**0.212***	0.130	0.164	0.144
	p	0.070	0.438	0.527	0.518	**0.018**	0.147	0.068	0.110
LDH	Rho	0.151	-0.033	**-0.255***	-0.120	0.039	0.262	0.138	0.127
	p	0.100	0.719	**0.005**	0.893	0.816	0.113	0.408	0.448
CRP	Rho	0.077	-0.174	**-0.571**	-0.098	0.259	-0.493	-0.106	-0.485
	p	0.760	0.489	**0.013**	0.699	0.393	0.087	0.731	0.093

Bold for significantly values (p value ≤ 0.05)

Interestingly, patients infected during the third wave showed the highest levels of SII, NLR, and PLR, while no significant differences were observed between the third and fifth waves, accounting for an important contribution to systemic alteration due to COVID-19. Consistent with these results, patients infected during the fifth wave showed also a lower percentage of HLA-DR+ monocytes. Therefore, the low expression of HLA-DR in monocytes and the high index values of inflammation in patients infected during the fifth wave showed a more important impairment of the immune response with respect to other waves (Lee et al., 2022).

Notably, we have also found systemic alterations in individuals with PASC. Regardless of the waves of the acute phase of COVID-19, both CD169+ and HLA-DR+CD169+ monocytes were found to be higher in PASCs than in HDs, supporting an immunological dysfunction as already delineated (Minutolo et al., 2023). The expression of HLA-DR+ appears to be different in different waves, especially in PASC who were infected in wave II and showed a lower percentage of HLA-DR+ monocytes than those in wave V. Moreover, PASC individuals infected in wave V showed higher percentages of circulating HLA-DR+CD169+ monocytes. Together, PASC individuals infected during waves V and VI showed elevated ISS and NLR values compared to other waves and healthy donors, underlining the potential impact of specific waves that showed altered inflammation values in the acute phase, such as the fifth. These results open new insight to evaluate the impact of different pandemic waves in the contribution of the systemic dysfunction of COVID-19 and its persistence in PASC individuals.

Although the wide number of both COVID-19 patients and PASC individuals enrolled, the limited number of samples when stratified for each wave is a limitation of the study. A wider range of cases would help a better understanding of the role of HLA-DR and CD169 markers with respect to clinical, and biochemical parameters and with post-COVID-19 symptoms. No effects of antiviral and corticosteroid treatments have

Table 7
Spearman correlation coefficient and P value of CD169 and HLA-DR with Inflammatory Indices parameters associated with disease severity in Acute COVID-19 and their association with the PASC phenotype.

INFLAMMATORY INDICES		COV				PASC			
		CD169 RMFI	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+CD169+ monocytes (%)	CD169 RMFI	CD169+ monocytes (%)	HLA-DR+ monocytes (%)	HLA-DR+CD169+ monocytes (%)
Charlson	Rho	-0.056	-0.085	-0.033	-0.074	**0.201***	0.116	0.147	0.149
	p	0.542	0.346	0.719	0.413	**0.028**	0.205	0.109	0.104
SII	Rho	-0.130	-0.122	-0.040	-0.104	**-0.259****	-0.133	0.038	-0.141
	p	0.148	0.169	0.654	0.243	**0.004**	0.148	0.678	0.123
NLR	Rho	-0.092	-0.135	-0.076	-0.104	-0.153	**-0.274****	-0.101	**-0.260****
	p	0.308	0.128	0.395	0.243	0.097	**0.002**	0.271	**0.004**

Bold for significantly values (p-value ≤ 0.05)

been demonstrated to modified the CD169 and HLA-DR expression in COVID-19 Acute patients, while monoclonal seems to be an impact on their expression, even if the number of patients treated with monoclonal was very low. This could depend on the type of drug that certainly has a more specific effect on immunologic modulations, and it will certainly be interesting to observe in larger case series the effects of this therapy.

In conclusion, confirming CD169 RMFI as a reliable marker of viral infection in different pandemic waves, herein we highlighted the presence of specific activated myeloid subpopulations in PASC individuals, suggesting a role of CD169 and HLA-DR expression in monocytes in both COVID-19 disease and in the chronic post-infection inflammation that persists in PASC, providing new tools to evaluate the impact of specific pandemic waves on systemic alterations in COVID-19 patients and PASC individuals, with the perspective to monitoring new variants and emerging respiratory viruses.

CRediT authorship contribution statement

Marialaura Fanelli: Formal analysis, Investigation, Methodology, Data curation, Validation, Writing – original draft, Writing – review & editing. **Vita Petrone:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Christian Marcchioni:** Data curation, Formal analysis, Methodology. **Rossella Chirico:** Formal analysis, Investigation. **Chiara Cipriani:** Writing – review & editing. **Luigi Coppola:** Resources, Data curation. **Vincenzo Malagnino:** Resources, Data curation. **Elisabetta Teti:** Resources, Data curation. **Chiara Sorace:** Resources, Data curation. **Marta Zordan:** Resources, Data curation. **Pietro Vitale:** Resources, Data curation. **Marco Iannetta:** Resources, Data curation. **Emanuela Balestrieri:** Writing – review & editing. **Guido Rasi:** Writing – review & editing. **Sandro Grelli:** Resources, Writing – review & editing. **Fabrice Malergue:** Resources, Writing – review & editing. **Loredana Sarmati:** Resources, Writing – review & editing. **Antonella Minutolo:** Conceptualization, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Claudia Matteucci:** Conceptualization, Validation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marialaura Fanelli reports financial support was provided by HERVCOV project funded by the HORIZONHLTH-2021-DISEASE G. A.101057302. Vita Petrone reports financial support was provided by HERVCOV project funded by the HORIZONHLTH-2021-DISEASE G. A.101057302. Chiara Cipriani reports financial support was provided by HERVCOV project funded by the HORIZONHLTH-2021-DISEASE G. A.101057302. Fabrice Malergue reports financial support was provided by Beckman Coulter LS.

Data availability

Data will be made available on request.

Acknowledgments

The study was supported by Beckman Coulter, which provided materials and reagents for CD169 analysis, and by facilities from the University of Rome "Tor Vergata". M.F., V.P. and C.C. were supported by the HERVCOV project funded by the HORIZONHLTH-2021-DISEASE project (Personalized medicine and infectious disease: understanding the individual host response to virus) of the European Commission under the Horizon Europe Framework Program. G.A.101057302.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2023.100215.

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