MAP-kinase mutations and aortic lesions are associated with distribution of circulating monocytes in histiocytosis

Histiocytosis represents a spectrum of rare myeloid neoplasms characterized by the accumulation and proliferation of CD68^{+} histiocytes, derived from the "monocyte/macrophage system," within organs.[1] These histiocytes, along with other inflammatory cells, contribute to disease symptoms. Recent research has underlined the major role of monocytes in the pathogenesis of histiocytosis.[2,3] Moreover, a specific monocyte subset distribution has been identified in histiocytosis, which is distinct from that in other myeloid neoplasms, and is associated with vascular lesions.[4] To characterize the impact of the clonal environment, the inflammatory syndrome, and vascular lesions on the distribution of monocyte subsets in histiocytosis, we compared this monocyte distribution in adult histiocytosis, giant cell arteritis (GCA), a vasculitis with an inflammatory pattern and frequent aortic lesions,[5] and healthy donors.

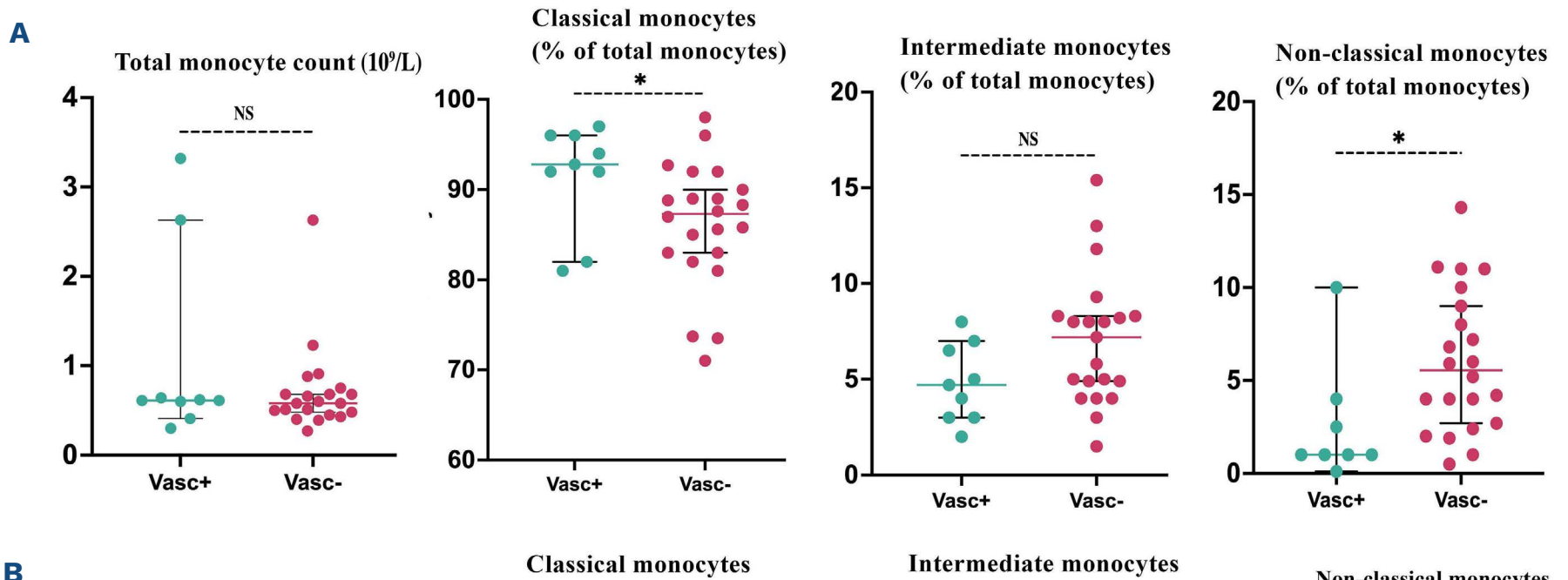
Peripheral blood samples were obtained from adult patients with histiocytosis (N=31), patients with GCA before starting steroid treatment (N=21), and healthy donors (N=21). The histiocytosis group, detailed in *Online Supplementary Table S1*, included 13 patients with Erdheim Chester disease (ECD), eight with Langerhans cell histiocytosis (LCH), four with Rosai-Dorfman disease (RDD), one with mixed ECD/LCH, two with juvenile xanthogranuloma, two with indeterminate cell histiocytosis, and one non-classifiable "L-group" histiocytosis. Research for *BRAF*^{V600E} and mitogen-activated protein kinase (MAP-kinase) pathway gene mutations was conducted on tissue biopsies using picodroplet digital polymerase chain reaction analysis and targeted next-generation sequencing, as previously reported.[6,7] The next-generation sequencing panel included: *AKT1, ALK, ARAF, ASXL1, BRAF, CALR, CBL, CDK4, CDKN1B, CDKN2A, CEBPA, CSF3R, CTNNB1, DNMT3A, EGFR, EZH2, FLT3, GATA2, GNA11, GNAQ, GNAS, HERC1, HRAS, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, KTM2D, MAML3, MAMLD1, MAP2K1, MAP2K2, MAP2K3, MAP2K4, MAP2K6, MAP3K1, MAP3K8, MAP3K9, MAP3K10, MAP3K19, MAP4K4, MAPK1, MAPK11, MAPK9, MPL, NF1, NOTCH1, NOTCH2, NPM1, NRAS, PDGFRA, PIK3CA, PP6C, PTEN, PTPN11, RAC1, RAF1, RIT1, RUNX1, SETBP1, SRSF2, STAG2, STK19, SYNGAP1, TAOK1, TAOK2, TET2, TP53, U2AF1, WT1,* and *ZRSR2*. Additionally, all histiocytosis patients were assessed for clonal hematopoiesis, either through bone marrow aspiration (N=24) or blood samples (N=7). The monocyte subset gating strategy, patients' characteristics and blood cell lineage comparison are reported in the *Online Supplementary Data*. The study followed the principles of the Declaration of Helsinki and was approved by the local ethics committee.

Compared to GCA patients, patients with histiocytosis had a decrease in the intermediate subset (median [interquartile range, IQR] 5.8% [4-8] *vs*. 7.8% [5.8-16]; *P*=0.04) (Figure 1A). Intermediate monocytes are considered proinflammatory monocytes because of their high production of inflammatory cytokines.[8] These findings, associated with the lower C-reactive protein (CRP) levels in patients with histiocytosis than in those with GCA (median [IQR]: 4 [3.7-11.5] *vs*. 75 [46-99] mg/L; *P*<0.0001), suggested that this specific monocyte distribution in histiocytosis was not related to inflammation. To look for an influence of clonal nature, the monocyte distribution in patients with histiocytosis was compared based on their molecular status and the presence of myeloid neoplasm driver genes within the bone marrow. Seven patients had *BRAF*^{V600E} mutations (4 ECD, 2 LCH, 1 mixed ECD/LCH), two had *MAP2K1* mutations (1 ECD, 1 RDD), and 13 had clonal hematopoiesis (mainly of *TET2*, *ASXL1*, and *DMNT3A* genes). Patients with MAP-kinase gene mutations had an increase in classical monocytes compared to patients with GCA and healthy donors (median [IQR]: 94% [89.5-96] *vs*. 86% [78.9-91.9] and *vs*. 85% [77-89]; *P*=0.02 and *P*=0.007, respectively), associated with a decrease in both intermediate monocytes (median [IQR]: 3.5% [2.25-4.75] *vs*. 7.8% [5.8-16] in patients with GCA and *vs*. 6.6% [4.4-13.3] in healthy donors; *P*=0.0017 and *P*=0.01, respectively), and in non-classical monocytes (median [IQR]: 2% [1-4.6] *vs*. 6.1% [4.3-10] for healthy donors; *P*=0.002) (Figure 1B). Patients with *BRAF*^{V600E}mutations exhibited a similar profile, with an increase in classical monocytes compared to healthy donors (median [IQR]: 94% [90-96] *vs*. 85% [77-89]; *P*=0.01), associated with a decrease in intermediate monocytes compared to patients with GCA and healthy donors (median [IQR]: 3.5% [2.7-4.2] *vs*. 7.8% [5.8-16] and *vs*. 6.6% [4.4-13.3]; *P*=0.005 and *P*=0.03, respectively), and in non-classical monocytes compared to healthy donors (median [IQR]: 2% [1-4] *vs*. 6.1% [4.3-10]; *P*=0.002) (Figure 1C). Classical monocytes, known for their role in phagocytosis,[9] harbor the *BRAF*^{V600E} mutation in histiocytosis.[2,3] The observed distribution echoes that found in previous studies,[10] confirming a distribution pattern similar to the one seen in chronic myelomonocytic leukemia and supporting the clonal nature of histiocytic disorders. There was no difference related to clonal hematopoiesis.

Finally, multiple linear regression analysis revealed an association between GCA and non-classical monocytes (β coefficient: -1.927; 95% confidence interval [95% CI]: -3.470 to -0.3839; *P*=0.0152), reaffirming the role of non-classical monocytes in pure vascular diseases.[11]

Regarding the other biological parameters, hemoglobin level was higher in patients with histiocytosis than in those with GCA (median [IQR]: 12.6 [11.5-14] g/dL *vs*. 11.0 [10.2-14.45] g/dL; *P*=0.0059). Conversely, the neutrophil count was lower in patients with histiocytosis than in GCA patients (median [IQR]: 4.4 [3.4-5.9] x10^9/L *vs*. 6.7 [4.6-8.2] x10^9/L; *P*=0.0327). These differences may support the inflammatory nature of GCA compared to histiocytosis.

Figure 1. The distribution of monocyte subsets in histiocytosis is associated with mitogen-activated protein-kinase mutation. (A) Monocyte subset distribution in patients with histiocytosis (N=31), patients with giant cell arteritis (GCA) (N=21) and healthy donors (HD) (N=21). (B) Monocyte subset distribution in mitogen-activated protein kinase-mutated patients (N=9), GCA patients (N=21) and HD (N=21). (C) Monocyte subset distribution in *BRAF*^{V600E}-mutated patients (N=7), GCA patients (N=21) and HD (N=21). NS: not statistically significant; MAPK: mitogen-activated protein kinase.



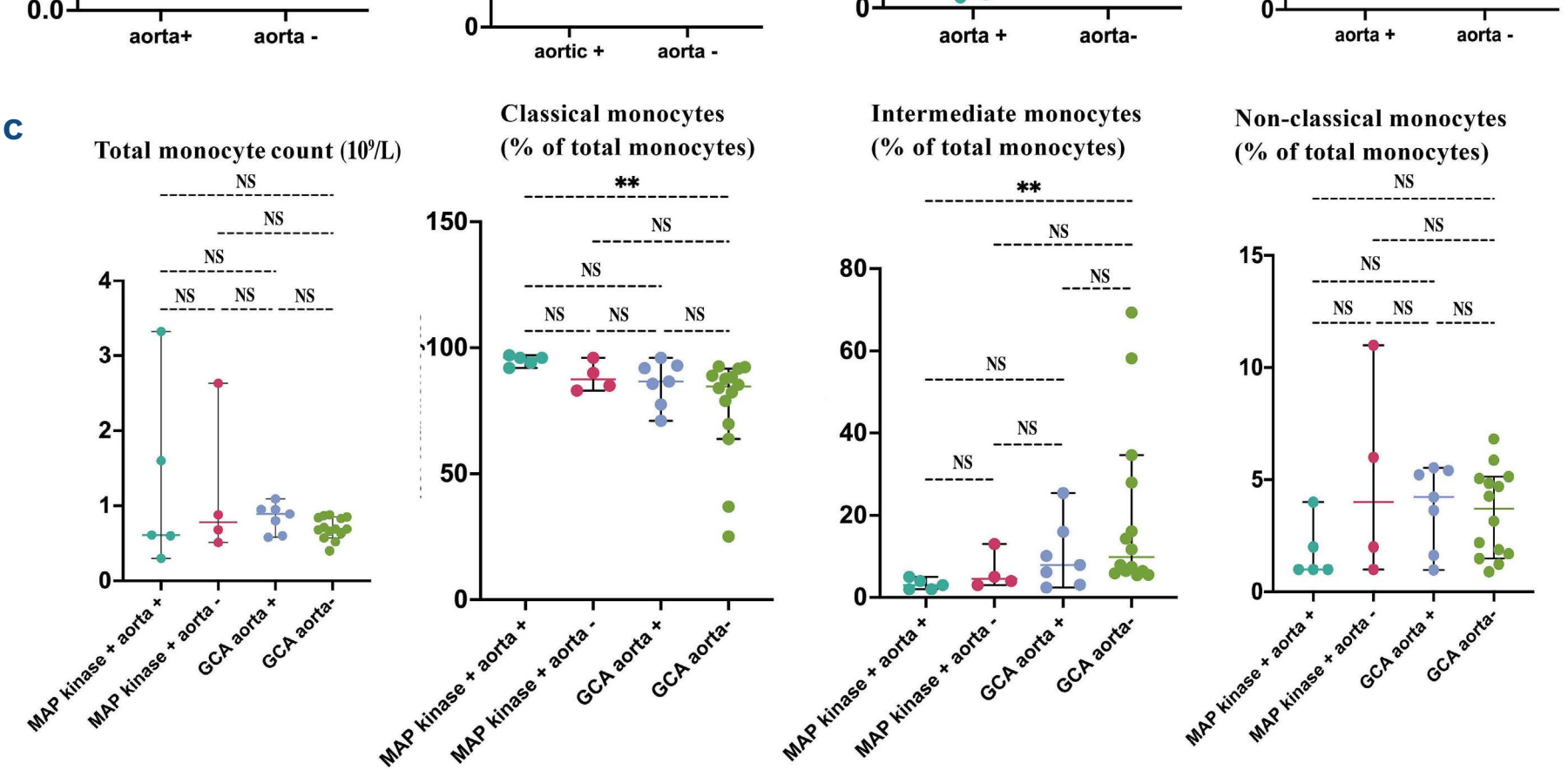


Figure 2. Histiocytosis patients with aortic lesions have a distinct monocyte subset distribution influenced by mitogen-activated protein kinase mutations. (A) Distinct monocyte subset distribution in histiocytosis patients according to aortic involvement. Vasc+ (N=9) are patients with vascular lesions and (Vasc-) (N=22) are patients without lesions. (B) No differences in monocyte subset distribution in patients with giant cell arteritis (GCA) independently of the presence of aortic lesions. Aorta + represents patients with aortic involvement (N=7) and aorta – represents those without aortic involvement (N=14). (C) Distinct monocyte subset distribution in patients with mitogen-activated protein kinase (MAP-kinase)-mutated histiocytosis with aortic involvement. MAP kinase + aorta + (N=5) are histiocytosis patients with MAP-kinase mutation; MAP kinase + aorta – (N=4) are histiocytosis patients with MAP-kinase mutation but no aortic lesions; GCA aorta + (N=7) are GCA patients with aortic lesions; GCA aorta – (N=14) are GCA patients without aortic lesions. NS: not statistically significant.

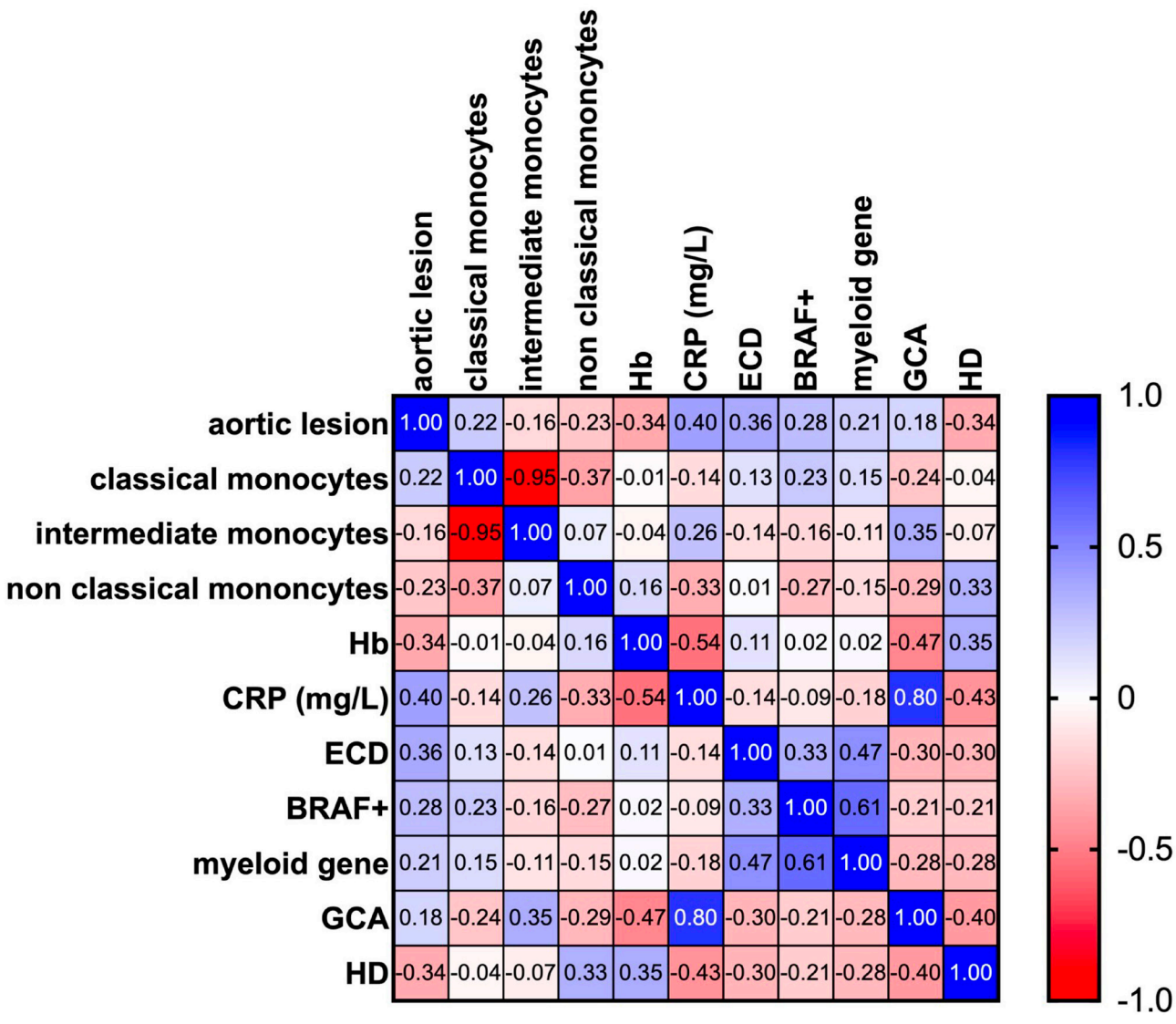


Figure 3. Heatmap of variables correlated with aortic lesions using a Pearson correlation model. Hb: hemoglobin; CRP: C-reactive protein; ECD: Erdheim Chester disease; GCA: giant cell arteritis; HD: healthy donors.

The second part of the study analyzed the distribution of the monocyte subsets according to the presence of aortic lesions. There were nine histiocytosis patients with aortic lesions 7 ECD, 1 ECD/LCH, 1 RDD) and seven GCA patients with aortic lesions at diagnosis compared to 22 histiocytosis patients and 14 GCA patients without aortic lesions. Histiocytosis patients with aortic lesions had an increase in classical monocytes (median [IQR]: 92% [87-96] *vs*. 87% [82-90]; *P*=0.04) and a decrease in non-classical monocytes compared with those without aortic lesions (median [IQR]: 1% [1-3.6] *vs*. 5.5% [2.6-9.2]; *P*=0.012) (Figure 2A). No difference was observed in GCA patients regarding the presence of aortic lesions (Figure 2B).

Because vascular involvement is correlated with MAP-kinase mutations in ECD,[12] we compared the histiocytosis patients with MAP-kinase mutations to GCA patients according to aortic phenotype. Histiocytosis patients with MAP-kinases mutations and aortic lesions (3 *BRAF* ECD, 1 *BRAF* ECD/LCH, and 1 *MAP2K1* RDD) had an increase in classical monocytes compared to GCA patients without aortic lesions (median [IQR]: 96% [93-96.5] *vs*. 84% [68-89]; *P*=0.072), and a decrease in intermediate monocytes compared to GCA patients without aortic lesions (median [IQR]: 3% [2.5-4.5] *vs*. 9.8% [6-15]; *P*=0.0042) (Figure 2C). These differences were not explained by a difference in CRP levels (median [IQR]: 1.4 [0.4-53] mg/L *vs*. 72 [47-83] mg/L; *P*=0.12).

MAP-kinase mutated patients, both with and without aortic lesions, had higher hemoglobin levels compared to GCA patients with aortic lesions (with lesions: median [IQR]: 13.5 [11.7-15.6] *vs*. 9.4 [8.9-10.2] g/dL; *P*=0.016, and without lesions: median [IQR]: 12.3 [11-13.7] *vs*. 9.4 [8.9-10.2] g/dL; *P*=0.025).

CRP levels were higher in GCA patients with aortic lesions than in MAP-kinase mutated patients, both with (median [IQR]: 116 [40-135] mg/L *vs*. 0.8 [0.4-53] mg/L; *P*=0.0154) and without (median [IQR]: 116 [40-135] mg/L *vs*. 3.5 [1.3-9.3] mg/L; *P*=0.0122) aortic lesions.

Finally, multivariate linear regression analysis revealed an association between aortic lesions and ECD (ß coefficient: 2.813; 95% CI: 0.1061-0.6274; *P*=0.0066) and with CRP levels (ß coefficient: 2.885; 95% CI: 0.001670-0.00921; *P*=0.0039); confirmed by a Pearson correlation model (Figure 3).

This study is the first investigation of the role of clonal environment, inflammatory patterns, and vascular lesions in the distribution of monocyte subsets in histiocytosis. To evaluate the impact of inflammation on monocyte subset distribution and aortic lesions, we compared histiocytosis with GCA (a large vessel vasculitis with frequent aortic involvement[5]). Our findings highlight the influence of the MAP-kinase pathway genes, especially *BRAF*^{V600E}, on the monocyte distribution in histiocytosis, supporting the clonal nature of histiocytosis,[1,13] and distinguishing

it from inflammatory diseases. The other biological parameters are in line with this finding, supporting the low inflammatory state in histiocytosis compared to that present in GCA.

Despite pathophysiological differences in aortic involvement (adventitia-isolated involvement in ECD[14] compared to total wall infiltration in GCA[5]), these findings underscore the significance of non-classical monocytes as potential markers of vascular aggression,[15] irrespective of the underlying condition. Additionally, they confirm the association between aortic lesions and ECD, in particular for patients with high CRP levels. Hence, despite the fact that the pathogenesis of histiocytic lesions is mostly driven by clonal signals, the association between high CRP levels and aortic lesions suggests a key role of inflammation in the development of vascular lesions, as in other inflammatory diseases with vascular tropism. However, our study has several limitations. First, previous treatments in histiocytosis patients may have influenced monocyte distribution. Secondly, clonal hematopoiesis, more frequent in histiocytosis[16] and GCA,[17] may have altered the monocyte subset distribution. The healthy donors were not age-matched with the patient populations, resulting in potential bias in the statistical analysis. Moreover, a study at a single-cell level may be informative about the mutational load and the role of each monocyte subset in histiocytosis, and the difference from a pure inflammatory disease. Nevertheless, this study is the first to shed light on the differences in monocyte subset distribution between histiocytosis and inflammatory vasculitis, confirming the role of the MAP-kinase pathway in monocyte distribution and also suggesting an association between the aortic phenotype and ECD patients with inflammatory syndrome.

In conclusion, MAP-kinase pathway gene mutations, mainly *BRAF*^{V600E}, influence monocyte distribution in histiocytosis, leading to a distinct distribution pattern associated with a low inflammatory state. Meanwhile, aortic lesions are associated with ECD.

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Disclosures

No conflicts of interest to disclose.

Contributions

JR, MS and BB designed the study. JG, HG, CR and MS performed the sample experiments. JR carried out the statistical analysis. JR, SA, JH, MP, FP, HG, MS and BB provided clinical expertise. JFE was responsible for histological samples including molecular analyses. All authors critically reviewed and approved the final manuscript.

Data-sharing statement

Complete data including flow cytometry analysis can be requested from the corresponding author.

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