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RESEARCH ARTICLE

Interleukin-6 transcripts up-regulation in lymph nodes from unicentric and multicentric Castleman disease

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Abstract

Introduction: Castleman disease (CD) represents a spectrum of heterogeneous lymphoproliferative disorders sharing peculiar histopathological features, clinically subdivided into unicentric CD (UCD) and multicentric CD (MCD) and presenting with variable inflammatory symptoms. Interleukin (IL)-6 and other cytokines play a major role in mediating CD inflammatory manifestations. Although the local microenvironment seems to be among the major sources of hypercytokinemia, the precise cellular origin of IL-6 production in CD is still debated.

Methods: A series of five nodal CD of different subtypes (one UCD, two idiopathic MCDs [iMCDs], one HIV-negative human herpesvirus 8 (HHV8)-associated MCD, and one HIV-positive HHV8-associated MCD) and a non-CD reactive control were tested using RNAscope analysis and a dual in situ hybridization (ISH)/immunohistochemistry technique, in order to quantify IL-6 expression and its spatial distribution. Quantitative analyses of in situ mRNA were performed on digitalized slides using the HISTOQUANT software (3DHISTECH) and differences between cases were evaluated by the Kruskal-Wallis test.

Results: RNA-ISH documented increased *IL-6* expression in all CD lymph nodes, independently from clinical and pathological subtypes, however, the highest levels were found in HHV8+ cases and statistically significant differences in IL-6 expression were found only between HHV8+ MCD and control case. Dual RNA-ISH for *IL6* coupled with immunohistochemistry analysis showed that IL-6 was overexpressed in CD31-positive endothelial cells in 5/5 CD tested cases but not in the control case.

Conclusion: Our findings suggest that nodal IL-6 expression seems to be significantly upregulated in HHV8+ MCD, but a trend toward increased nodal IL-6 expression was noticed also in UCD and iMCD-not otherwise specified. CD31+ endothelial

cells probably represent one of the major sources of IL-6 production in the nodal microenvironment.

KEYWORDS
HHV8, IL-6 transcripts, multicentric Castleman disease, RNA-ISH, unicentric Castleman disease

1 | INTRODUCTION

Castleman disease (CD), recently included by the World Health Organization (WHO) lymphoma classification among the tumor-like lesions with B-cell predominance, identifies a spectrum of heterogeneous lymphoproliferative disorders sharing peculiar histopathological features [1].

Clinically, CD is distinguished into unicentric CD (UCD) and multicentric CD (MCD) [2].

UCD usually involves a single lymph node site, with no/minimal and/or local symptoms, excisional surgery being often curative [3]. MCD presents as a systemic lymphadenopathy, with constitutional symptoms, systemic inflammation, and multi-system organ dysfunction often requiring immunotherapy and/or chemotherapy [4]. By etiologic driver MCD is subdivided into human herpesvirus 8 (HHV8)-associated MCD with or without HIV coinfection (HIV ±); polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes (POEMS)-associated MCD; and idiopathic MCD (iMCD) furtherly distinguished into iMCD-not otherwise specified (iMCD-NOS) [5], often associated with thrombocytosis and hypergammaglobulinemia, and iMCD-TAFRO (thrombocytopenia, anasarca, reticulin fibrosis, renal dysfunction, and organomegaly) [6].

Histopathologic features of CD vary from a hyaline-vascular (HV)/hypervascular (HyperV) subtype, a plasma cell (or plasmacytic) subtype, and mixed forms that are in between [7, 8].

The vast majority of UCDs have hyaline vascular histology with follicular dendritic cells-enriched atretic follicles, fused mantle zones, and prominent interfollicular vascularity with hyalinization [9]. MCD may also contain atretic follicles but is usually associated with hyperplastic ones, increased interfollicular vascularity, and variable plasmacytosis, resulting in hypervascular, plasmacytic, or mixed patterns [10, 11].

The CD histopathologic features are well known, but the putative cellular and molecular pathways responsible for the heterogeneous CD clinical behavior and clinical course remain poorly understood [12–14]. Various studies pointed out the pathogenetic role of nodal microenvironment in cytokines production [15]. Indeed, whereas UCD is likely mediated by dysregulation of lymph node stromal cells, MCD is characterized by a systemic hypercytokinemia that includes interleukin (IL)-6 [16], vascular endothelial growth factor (VEGF) and other chemokines [17]. The source of hypercytokinemia depends on MCD subtype: in HHV8-associated MCD, hypercytokinemia is driven by the virus that produces a viral IL-6 (vIL-6), homologous of the human IL-6 (hIL-6) [18–20], that exerts its effect by binding the hIL-6 receptor and upregulating hIL-6 production; in POEMS-associated MCD, hypercytokinemia is related to monoclonal plasma cells. IL-6 seems to be the most common pathological driver also in the iMCD in which most of the CD systemic symptoms are linked to IL-6 hyperfunction [21]. The crucial pathogenetic role of cytokines in CD, particularly including IL-6, is corroborated by the reduction of symptoms in up to half of patients with iMCD treated with targeting IL-6 monoclonal antibody tocilizumab or siltuximab [22, 23].

In spite of this evidence, data concerning the IL-6 precise pathogenetic contribution and source of production in CD are limited and partially contrasting. Two previous studies analyzed the lymph node transcriptome of UCD and MCD, providing different results about IL-6 transcription/expression in CD nodal microenvironment. Wing et al. [24] performed a multi-platform analysis using targeted RNA sequencing, RNA in situ hybridization (ISH), and adaptative immune receptor rearrangements profiling in a series of UCD and MCD, demonstrating IL-6 upregulation in MCD only.

In contrast, based on whole exome sequencing and immunohistochemistry approach, Horna et al. [25] reported upregulation of CXCL13 but not IL-6, in both UCD and iMCD. With this background, we employed RNAscope analysis [26] coupled with a dual ISH/IHC technique in a series of nodal CD of different subtypes, in order to quantify IL-6 expression and its spatial distribution.

2 | METHODS

Five CD cases (one UCD, two iMCDs, one HIV-negative HHV8-associated MCD, and one HIV-positive HHV8-associated MCD) and a non-CD reactive control were tested to assess *IL6* mRNA (RNAScope, Biotechne) expression and quantification. Formalin-fixed paraffin-embedded (FFPE) tissues were utilized for RNAscope and immunohistochemistry (IHC) analysis. The human IL-6 probe hybridization (Cod. 310371) was performed using RNAscope 2.5 HD Detection Reagent-BROWN (Advanced Cell Diagnostic) in accordance with the manufacturer's protocol. A dual ISH-IHC protocol was validated to simultaneously stain selected FFPE tissue slides with immunohistochemistry antibodies and RNAscope probes.

Human tissue sections were deparaffinized, rehydrated, and unmasked using Novocastra Epitope Retrieval Solutions at pH = 6 and pH = 9 in a thermostatic bath at 98°C for 30 min. Subsequently, the sections were brought to room temperature and washed in phosphate-buffered saline (PBS). After neutralization of the endogenous peroxidase with 3% H_2O_2 and Fc blocking by 0.4% casein in PBS (Novocastra), the sections were incubated with antibodies. For

multiple-marker immunostaining, sections were subjected to sequential rounds of single-marker immunostaining, and the binding of the primary antibodies was revealed by the use of specific secondary antibodies conjugated with different enzymes. The following primary antibodies were used for IHC on human tissues: rabbit anti-human CD3 (1:100, pH = 9, Abcam), mouse anti-human CD68R/PGM1 (clone 514H12, ready to use, ph = 9, Leica Biosystems), CD138 and mouse anti-human CD31 (clone JC70A, ready to use, ph = 9, Leica Biosystems). Double IHC staining was performed by applying SignalStainBoost IHC Detection rabbit (cod. #18653, Cell Signaling Technology) alkaline phosphatase-conjugated produced in horse and Vulcan Fast Red as substrate chromogen; and SignalStainBoost IHC Detection mouse (cod. #8125S, Cell Signaling Technology) horseradish peroxidase (HRP)-conjugated produced in goat and PolyDetector HRP Green as substrate chromogen. Slide digitalization was performed using an Aperio CS2 digital scanner (Leica Biosystems) with the ImageScope software (Aperio ImageScope version 12.3.2.8013, Leica Biosystems). Quantitative analyses of in situ mRNA were performed by calculating the average percentage of positive cells in five non-overlapping areas at medium-power magnification (x200) using the HISTOQUANT software (3DHISTECH) and the output was expressed as "AREA %". Differences between cases were evaluated by the Kruskal-Wallis test, a two-sided p-value < 0.05 was considered statistically significant

3 | RESULTS

3.1 | Clinicopathologic characteristics of cases

Five CD cases were extracted from the files of the Pathologic Anatomy Unit of the University of Pavia/Foundation IRCCS Policlinico "San Matteo," Pavia for IL-6 RNA-ISH analysis, based on the availability of histological samples obtained in the last two years. Selected cases included one UCD, two iMCDs, one HHV8+/HIV- MCD, and one HHV8+/HIV+ MCD. Histological CD diagnoses on nodal samples were made and reviewed by three expert hematopathologists (Marco Lucioni, Marcello Gambacorta, and Marco Paulli) according to the Castleman Disease Collaborative Network (CDCN) consensus criteria [2, 5] and the recommendations contained in the most recent WHO classification of the tumors of hematolymphoid tissues [1]. Infectious, malignant, and autoimmune disorders that can mimic iMCD were excluded based on the correlation with clinical data.

The main clinical and pathological features of selected cases are detailed in Table 1.

A non-specific reactive lymph node was also included in the study to compare IL6 expression between CD a non-CD lymph nodes.

3.2 | RNA-ISH analysis

RNA-ISH, which is more sensitive and specific than IHC, was used to identify the lymph node structures and cells expressing *IL6*. Quantification of *IL6* mRNA was performed on 5 fields at 200x magnification (Table S1) and revealed an increased *IL6* expression in all tested CD lymph nodes (median IL6 expression area % ranging between 0.25 and 0.77 in each individual case; mean IL6 expression area % ranging between 0.28 and 0.86 in each individual case) in comparison with reactive control lymph node (median IL6 expression area % 0.13; mean IL6 expression area % 0.11). Increased *IL6* expression was observed independently from clinical and pathological subtypes (Figure 1), however, the highest levels were found in HHV8+ cases, whereas HV-UCD and iMCD hyperV showed lower *IL6* expression. Post-hoc analyses were also conducted, showing statistically significant differences ($p = 0.0005$) in IL-6 expression only between HHV8 positive cases and control (Figure S1).

Serum IL-6 data were available in four out of five cases. Comparison between serum IL-6 and the median *IL6* RNA-ISH expression in lymph nodes confirmed that HHV8+ cases had a higher value of both serum IL-6 and nodal *IL6* mRNA.

Since RNA ISH maintains the tissue histomorphological tissues, we assessed IL-6 expression in different nodal regions, including the germinal center, mantle zone, and interfollicular areas. Based on this approach, we observed *IL6* mRNA expression in scattered cells, mostly dispersed in the interfollicular areas, both in UCD and MCD cases. In particular, the cells expressing *IL6* seemingly tracked along hypervascular areas in the nodal interfollicular compartment.

3.3 | Dual RNAscope/IHC analysis

In order to explain the peculiar histomorphological pattern of *IL6* mRNA analysis, we conducted dual RNA-ISH/IHC stainings to identify the nodal cellular subpopulations expressing IL-6 [26]. The dual staining did not reveal significant co-expression of *IL6* with T-cells (assessed by CD3), macrophages (evaluated through CD68/PGM1), or plasma cells (highlighted by CD138). On the other hand, dual RNA-ISH for *IL6* and IHC for CD31 staining showed that IL-6 was overexpressed in CD31-positive endothelial cells in 5/5 CD tested cases but not in the control case, where *IL6* mRNA and CD31 protein did not colocalize (Figure S2). These observations suggest that endothelial cells may be the major local source of IL-6 in both UCD and MCD lymph nodes (Figure 2).

4 | DISCUSSION

Several studies highlighted the central pathogenetic role of IL-6 and other cytokines in mediating the inflammatory manifestations of CD [27, 28]. In particular, high levels of serum IL-6 in patients with iMCD seem to be associated with increased disease activity [5, 29]. In transgenic mice, overexpression of IL-6 or the HHV8-encoded vIL-6 leads to the development of clinic-pathologic syndromes reminiscent of MCD [30]. Most importantly, antibodies that block IL-6 (siltuximab) [23] or its receptor (tocilizumab) [22] proved to be effective in reducing symptoms in iMCD patients, and now represent frontline

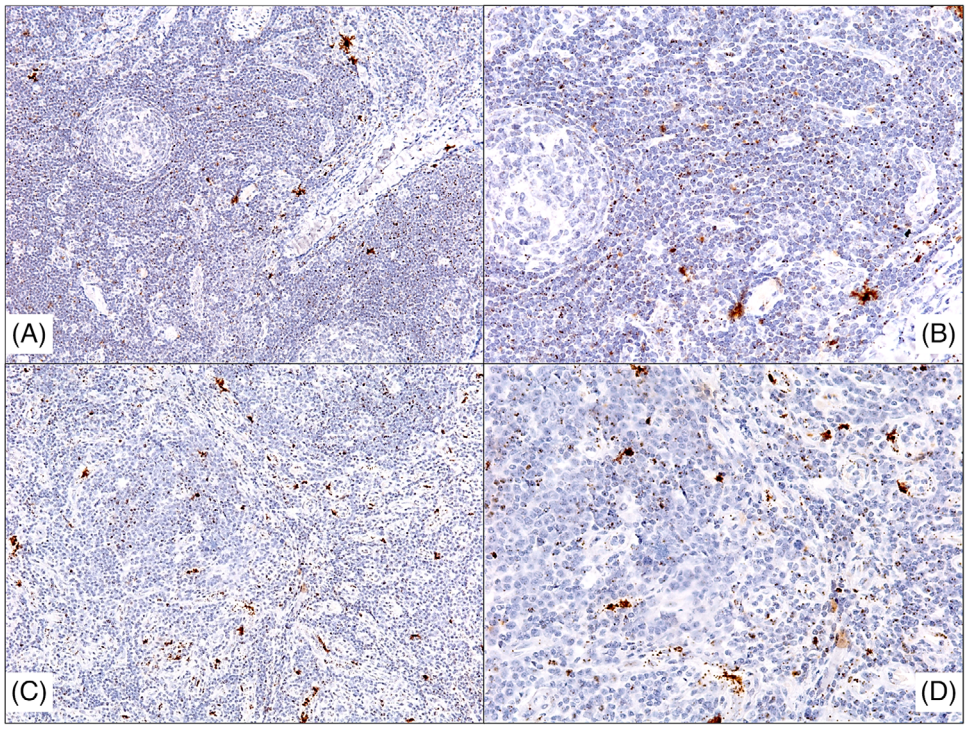

FIGURE 1 Interleukin (IL)-6 expression in an idiopathic multicentric Castleman disease (iMCD) case with HyperV histology at lower (10x) (A) and higher (20x) magnification (B); and in human herpesvirus 8 (HHV8)+HIV-MCD case with mixed histology (C, 10x; D 20x).

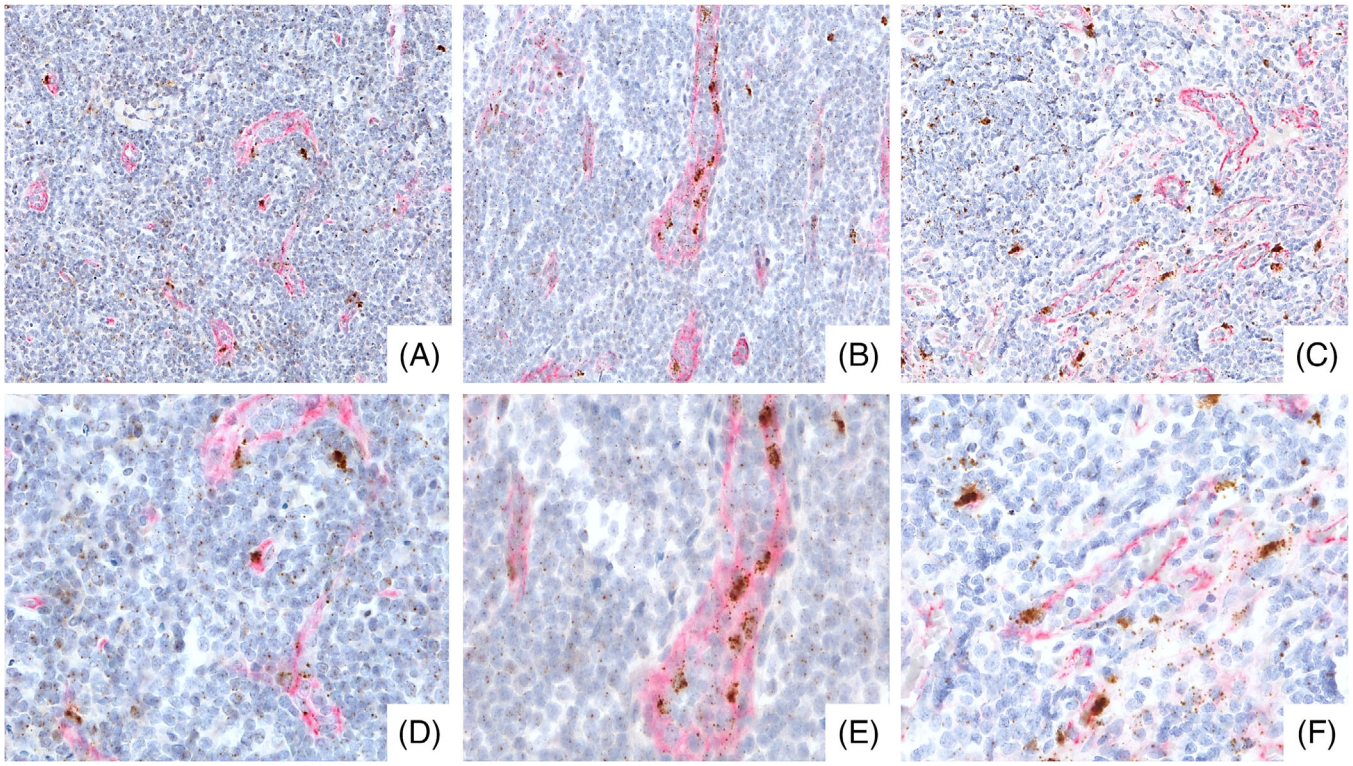

FIGURE 2 Dual RNAscope/immunohistochemistry (IHC) showing that CD31+cells (red) are the major source of IL-6 (brown) in Castleman disease (CD); unicentric CD hyaline-vascular (UCD HV) (A, 20x); idiopathic multicentric CD (iMCD) mixed histology (B, 20x); human herpesvirus 8 (HHV8)+HIV+MCD mixed histology (C, 20x); higher magnification of UCD HV (D, 40x), iMCD mixed (E, 40x), and HHV8+HIV+MCD mixed (F, 40x).

TABLE 1 Main clinical and histological features.

N	Age	Sex	UCD versus MCD	Histology	Increase in CD8+cells	Serum IL-6 (pg/mL)	HHV-8	HIV	Therapy	Outcome
1	75	M	MCD	CD mixed	No	67.7	+	-	Rituximab	Dead for complications disease-related
2	43	M	UCD	CD HV	Yes	6.02	-	-	Surgery	Alive
3	57	M	MCD	CD HyperV	No	n.a.	-	-		LFU
4	54	M	MCD	CD mixed	Yes	42.7	+	+	Doxorubicin +Rituximab	Alive
5	56	F	MCD	CD mixed	Yes	24.7	-	-	Siltuximab	Alive

Abbreviations: CDHV, Castleman disease hyaline-vascular type; CDHyperV, multicentric Castleman disease hypervascular type; HHV-8, human herpesvirus 8; IL, interleukin; LFU, lost to follow-up; MCD, multicentric Castleman disease; UCD, unicentric Castleman disease.

therapies. Although data accumulated indicating the local microenvironment among the major sources of hypercytokinemia, the precise cellular source of IL-6 production in CD is still debated. Investigations using ISH and or quantitative real-time polymerase chain reaction to detect *IL6* overexpression in lymph nodes or lung biopsies involved by iMCD, demonstrated increased *IL6* mRNA expression only in a minority of cases, suggesting that IL6 could be produced outside the affected organ [31, 32]. Two recent studies focusing on CD transcriptome and cytokine microenvironment provided contrasting results about IL-6 increased expression in CD lymph nodes. Wing et al. [24] reported nodal upregulation of IL-6 expression in MCD, whereas analysis of cytokine transcripts by Horna et al. [25] showed upregulation of CXCL13 but not IL-6 in UCD and iMCD. Spatially resolved in situ analysis of biomarkers is highly desirable in molecular pathology because it allows the examination of biomarkers' status within the histopathological context of clinical specimens [33]. On such bases, we employed the transcriptomic analysis in order to evaluate the expression and quantification of *IL6* RNA-ISH in nodal biopsies from different CD subtypes including HHV8-related form. RNA-ISH was used to assess *IL6* expression in respectively five lymph nodes encompassing different clinico-pathological subtypes of CD (HV-UCD, HyperV-iMCD, iMCD-mixed, HHV8+/HIV+ MCD mixed, HHV8+/HIV- MCD mixed) and one control reactive hyperplastic lymph node. In contrast with Horna et al. [25] our findings seem to confirm the observation by Wing et al [24] documenting IL-6 up-regulation in CD lymph nodes in comparison with the control case.

Nevertheless, whereas Wing et al [24] demonstrated IL-6 increased expression in MCD cases only, we observed a variable increased IL-6 expression in all tested CD cases, irrespectively from their clinical presentation (unicentric vs. multicentric) and histologic subtype, although the comparison with control case IL-6 expression reached the threshold of statistical significance only for HHV8+ cases. The variability in *IL6* mRNA in situ analysis results between different studies could be ascribed in part to the different used methods, in part to the fact that RNA stainability depends on the RNA quality at the time of testing, the latter being related to the age and storage conditions of paraffin blocks. In order to optimize the probability of detecting good-quality RNA, actually we restricted our analyses to the cases with histological samples obtained in the last two years.

Many types of cells (both immune and stromal cells) but mainly T cells and macrophages, can produce Human (h)IL-6. Previous studies using immunohistochemistry on CD lymph node biopsies alternatively suggested IL-6 expression in germinal center B cells, plasma cells, monocytes, follicular dendritic cells, and endothelial cells [24].

Since *IL6* mRNA expression was observed in the interfollicular areas of our CD cases, we performed IHC/ISH dual stainings using various cellular markers including CD3, CD68R CD138, and CD31, in order to detect the different cellular subpopulations that might be responsible for IL-6 release in nodal microenvironment. Based on this approach we were able to evidentiate that IL-6 up-regulation was predominantly detectable in CD31+ endothelial/lymphatic cells, including the section from the case of HHV8-associated CD. Similar observations were reported in previous studies, that suggested that vasculature-associated cells may be a significant source of IL-6 production in enlarged CD lymph nodes [24]; however, we cannot exclude that endothelial cells may play a role in IL-6 secretion even in other (extra-nodal) compartments. We noticed that the median IL-6 expression value was higher in HHV8+ cases, representing the only subset in which IL-6 upregulation resulted to be statistically significant in comparison with the control case. It might be postulated that such an increase in HHV8-related CD could be ascribed to the release of vIL-6. The latter can stimulate the production of hIL-6 with a paracrine mechanism: similarly to hIL-6, vIL-6 up-regulates VEGF that can in turn induce hIL-6 expression by endothelial cells [7]. A positive correlation between IL-6 nodal tissue expression and IL6 serum levels was also found in HHV8+ CD cases. Since IL-6 is a growth factor for plasma cells and can also induce VEFG secretion, its local upregulation may provide at least in part a biological explanation for the histological changes associated both with HV/hyperV and PC or mixed CD subtypes, via paracrine mechanisms.

Recently we reported some peculiar modifications in the T-cell subset distribution occurring in CD, independently from clinic-pathologic

subtype [34]. In particular, we noticed a decreased CD4/CD8 ratio due to increased CD8+ T cells and a decreased number of FOXP3 regulatory T (Treg) cells. IL-6, often in combination with multiple other cytokines, contributes to the generation and differentiation of CD8+ T cell subsets [35].

Some recent studies about the neoplastic microenvironment in murine models confirmed that IL-6 might mobilize a T-cell antineoplastic immune response, promoting differentiation of naive CD8+ T cells in specific CD8+ effectors [36].

With a similar mechanism we can hypothesize that increased IL-6 expression in CD nodal microenvironment could favor the expansion of CD8+ T cells subpopulations that we previously detected in a subset of CD cases. Actually, 3/5 cases we analyzed for IL-6 transcripts expression in nodal biopsies showed a concurrent increased population of CD8+ T-cells, including 1 HV-UCD and 1 mixed MCD, in addition to the HHV8+/HIV+ MCD. IL-6 plays a role also in the commitment of Treg cells [37]. Although the functional consequence of IL-6 sensing by Treg cells seems to be context-dependent, overall IL-6 acts as a suppressor of FOXP3 and other core signature genes of Treg cells by transcriptional and post-transcriptional mechanisms [38].

Our study has some limitations. The major limitation is represented by the particularly small number of tested cases. This is due to the fact that, in order to optimize the probability of detecting good quality RNA and the reliability of *IL6* RNA-ISH, we restricted our analyses only to patients with histological samples obtained in the last two years and processed at a single laboratory. This approach did not allow us to include cases of iMCD-TAFRO and of so-called idiopathic plasmacytic lymphadenopathy (IPL). iMCD-TAFRO is a very rare variant, but some recent studies suggest that VEGF, and the interplay of VEGF and IL-6 in concert, rather than IL-6 as a single cytokine, may be drivers for iMCD-TAFRO pathophysiology [39]. Therefore we can not exclude different results in IL6 expression in this subset of patients. IPL is an entity characterized by polyclonal hypergammaglobulinemia, plasmacytic/mixed type lymph node histopathology, and thrombocytosis, that was most recently proposed to represent a distinct subtype of iMCD-NOS [40]. Patients with IPL also showed a significantly higher increase in IL-6 level and inflammatory state but longer overall survival if compared with other iMCD-NOS patients [41]. These observations suggest that IL-6 may be a disease driver in this disease subgroup and IL-6 expression analysis in lymph node samples would be useful to confirm the role of IL-6 in IPL and its position in the entire iMCD spectrum.

5 | CONCLUSION

In conclusion, our findings seem to suggest that nodal IL-6 expression is significantly upregulated in HHV8+ MCD, but a trend toward increased nodal IL-6 expression was noticed also in UCD and iMCD-NOS. The CD31+ endothelial cells probably represent one of the major sources of IL-6 production in the nodal microenvironment. Additional studies, based on a spatially resolved approach to the analysis of the upregulated genetic pathways, are needed to better understand the role of IL-6 and other cytokines in the pathogenesis of CD.

AUTHOR CONTRIBUTIONS

Conceptualization: Marco Paulli, Marco Lucioni, and Claudio Tripodo. *Formal analysis*: Gaia Morello and Sara Fraticelli. *Histologic diagnoses*: Marco Lucioni, Marcello Gambacorta, and Marco Paulli. *Investigation*: Gaia Morello, Sara Fraticelli, Caterina Cristinelli, Giuseppe Neri, Erica Travaglino, Marco Minetto, Francesca Antoci, and Paolo Libretti. *Data curation*: Sara Fraticelli, Gaia Morello, Giuseppe Neri, Caterina Cristinelli, and Marco Minetto. *Writing—original draft preparation*: Marco Lucioni, Sara Fraticelli, and Gaia Morello. *Writing—review and editing*: Marco Lucioni, Caterina Cristinelli, Sara Fraticelli, Luca Arcaini, and Marco Paulli. *Supervision*: Marco Paulli, Luca Arcaini, Marcello Gambacorta, and Claudio Tripodo. All authors have read and agreed to the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Luca Arcaini: Honoraria: EUSA Pharma, Novartis. Participation on a Data Safety Monitoring Board or Advisory Board Roche, Janssen-Cilag, Verastem, Incyte, EUSA Pharma, Celgene/Bristol Myers Squibb, Kite/Gilead, ADC Therapeutics, Novartis; Support for attending meetings and/or travel: Roche. Marco Paulli: Honoraria: Recordati Rare diseases.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and approved by the local Ethics Committee.

PATIENT CONSENT STATEMENT

The authors have confirmed clinical trial registration is not needed for this submission.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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REFERENCES

1. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. Leukemia. 2022;36(7):1720–48. https://doi.org/10.1038/s41375-022-01620-2. Epub 2022 Jun 22. Erratum in: Leukemia. 2023 Sep;37(9):1944-1951.
2. Dispenzieri A, Fajgenbaum DC. Overview of Castleman disease. Blood. 2020;135(16):1353–64.
3. van Rhee F, Oksenhendler E, Srkalovic G, Voorhees P, Lim M, Dispenzieri A, et al. International evidence-based consensus diagnostic and treatment guidelines for unicentric Castleman disease. Blood Adv. 2020;4(23):6039–50.
4. Oksenhendler E, Boutboul D, Fajgenbaum D, Mirouse A, Fieschi C, Malphettes M, et al. The full spectrum of Castleman disease: 273 patients studied over 20 years. Br J Haematol. 2018;180(2):206–16.
5. Fajgenbaum DC, Uldrick TS, Bagg A, Frank D, Wu D, Srkalovic G, et al. International, evidence-based consensus diagnostic criteria for HHV-8-negative/idiopathic multicentric Castleman disease. Blood. 2017;129(12):1646–57.
6. Igawa T, Sato Y. TAFRO syndrome. Hematol Oncol Clin North Am. 2018;32(1):107–18.
7. Carbone A, Borok M, Damania B, Gloghini A, Polizzotto MN, Jayanthan RK, et al. Castleman disease. Nat Rev Dis Primers. 2021;7(1):84.
8. Wu D, Lim MS, Jaffe ES. Pathology of Castleman disease. Hematol Oncol Clin North Am. 2018;32(1):37–52.
9. Nishimura MF, Nishimura Y, Nishikori A, Maekawa Y, Maehama K, Yoshino T, et al. Clinical and pathological characteristics of hyaline-vascular type unicentric Castleman disease: a 20-year retrospective analysis. Diagnostics. 2021;11(11):2008.
10. Liu AY, Nabel CS, Finkelman BS, Ruth JR, Kurzrock R, van Rhee F, et al. Idiopathic multicentric Castleman's disease: a systematic literature review. Lancet Haematol. 2016;3(4):e163–75.
11. Hoffmann C, Hentrich M, Tiemann M, Rosenwald A, Weber F, Willenbacher W, et al. Recent advances in Castleman disease. Oncol Res Treat. 2022;45(11):693–704.
12. Fajgenbaum DC, Shilling D. Castleman disease pathogenesis. Hematol Oncol Clin North Am. 2018 ;32(1):11–21.
13. Fajgenbaum DC, van Rhee F, Nabel CS. HHV-8-negative, idiopathic multicentric Castleman disease: novel insights into biology, pathogenesis, and therapy. Blood. 2014;123(19):2924–33.
14. El-Osta HE, Kurzrock R. Castleman's disease: from basic mechanisms to molecular therapeutics. Oncologist. 2011;16(4):497–511.
15. Chang JE, Turley SJ. Stromal infrastructure of the lymph node and coordination of immunity. Trends Immunol. 2015;36(1):30–39.
16. Leger-Ravet MB, Peuchmaur M, Devergne O, Audouin J, Raphael M, Van Damme J, et al. Interleukin-6 gene expression in Castleman's disease. Blood. 1991;78(11):2923–30.
17. Nishi J, Maruyama I. Increased expression of vascular endothelial growth factor (VEGF) in Castleman's disease: proposed pathomechanism of vascular proliferation in the affected lymph node. Leuk Lymphoma. 2000;38(3-4):387–94.
18. Aoki Y, Jaffe ES, Chang Y, Jones K, Teruya-Feldstein J, Moore PS, Tosato G. Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. Blood. 1999;93(12):4034–43.
19. Parravicini C, Corbellino M, Paulli M, Magrini U, Lazzarino M, Moore PS, Chang Y. Expression of a virus-derived cytokine, KSHV vIL-6, in HIV-seronegative Castleman's disease. Am J Pathol. 1997;151(6):1517–22.
20. St Paul M, Saibil SD, Lien SC, Han S, Sayad A, Mulder DT, et al. IL6 induces an IL22$^+$ CD8$^+$ T-cell subset with potent antitumor function. Cancer Immunol Res. 2020;8(3):321–33.
21. Uciechowski P, Dempke WCM. Interleukin-6: a masterplayer in the cytokine network. Oncology. 2020;98(3):131–37.
22. Matsuyama M, Suzuki T, Tsuboi H, Ito S, Mamura M, Goto D, et al. Anti-interleukin-6 receptor antibody (tocilizumab) treatment of multicentric Castleman's disease. Intern Med. 2007;46(11):771–74.
23. van Rhee F, Wong RS, Munshi N, Rossi JF, Ke XY, Fosså A, et al. Siltuximab for multicentric Castleman's disease: a randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2014;15(9):966–74. https://doi.org/10.1016/S1470-2045(14)70319-5. Epub 2014 Jul 17. Erratum in: Lancet Oncol. 2014 Sep;15(10):417.
24. Wing A, Xu J, Meng W, Rosenfeld AM, Li EY, Wertheim G, et al. Transcriptome and unique cytokine microenvironment of Castleman disease. Mod Pathol. 2022;35(4):451–61.
25. Horna P, King RL, Jevremovic D, Fajgenbaum DC, Dispenzieri A. The lymph node transcriptome of unicentric and idiopathic multicentric Castleman disease. Haematologica. 2023;108(1):207–18.
26. Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn. 2012;14(1):22–29.
27. Yoshizaki K, Murayama S, Ito H, Koga T. The role of interleukin-6 in Castleman disease. Hematol Oncol Clin North Am. 2018;32(1):23–36.
28. Pierson SK, Stonestrom AJ, Shilling D, Ruth J, Nabel CS, Singh A, et al. Plasma proteomics identifies a 'chemokine storm' in idiopathic multicentric Castleman disease. Am J Hematol. 2018;93(7):902–12.
29. van Rhee F, Voorhees P, Dispenzieri A, Fosså A, Srkalovic G, Ide M, et al. International, evidence-based consensus treatment guidelines for idiopathic multicentric Castleman disease. Blood. 2018;132(20):2115–24.
30. Katsume A, Saito H, Yamada Y, Yorozu K, Ueda O, Akamatsu K, et al. Anti-interleukin 6 (IL-6) receptor antibody suppresses Castleman's disease like symptoms emerged in IL-6 transgenic mice. Cytokine. 2002;20(6):304–11.
31. Otani K, Inoue D, Fujikura K, Komori T, Abe-Suzuki S, Tajiri T, et al. Idiopathic multicentric Castleman's disease: a clinicopathologic study in comparison with IgG4-related disease. Oncotarget. 2018;9(6):6691–706.
32. Kinugawa Y, Uehara T, Iwaya M, Asaka S, Kobayashi S, Nakajima T, et al. IL-6 expression helps distinguish Castleman's disease from IgG4-related disease in the lung. BMC Pulm Med. 2021;21(1):219.
33. Tripodo C, Zanardi F, Iannelli F, Mazzara S, Vegliante M, Morello G, et al. A spatially resolved dark- versus light-zone microenvironment signature subdivides germinal center-related aggressive B cell lymphomas. iScience. 2020;23(10):101562.
34. Fraticelli S, Lucioni M, Neri G, Marchiori D, Cristinelli C, Merli M, et al. T-Cells subsets in Castleman disease: analysis of 28 cases including unicentric, multicentric and HHV8-related clinical forms. Int J Mol Sci. 2023;24(9):7813.
35. Korn T, Hiltensperger M. Role of IL-6 in the commitment of T cell subsets. Cytokine. 2021;146:155654.
36. Fisher DT, Appenheimer MM, Evans SS. The two faces of IL-6 in the tumor microenvironment. Semin Immunol. 2014;26(1):38–47.
37. Maharaj K, Uriepero A, Sahakian E, Pinilla-Ibarz J. Regulatory T cells (Tregs) in lymphoid malignancies and the impact of novel therapies. Front Immunol. 2022;13:943354.
38. Gao Z, Gao Y, Li Z, Chen Z, Lu D, Tsun A, Li B. Synergy between IL-6 and TGF-β signaling promotes FOXP3 degradation. Int J Clin Exp Pathol. 2012;5(7):626–33.

39. Srkalovic G, Nijim S, Srkalovic MB, Fajgenbaum D. Increase in Vascular Endothelial Growth Factor (VEGF) Expression and the Pathogenesis of iMCD-TAFRO. Biomedicines. 2024;12(6):1328. https://doi.org/10.3390/biomedicines12061328
40. Nishikori A, Nishimura MF, Nishimura Y, Otsuka F, Maehama K, Ohsawa K, et al. Idiopathic Plasmacytic Lymphadenopathy Forms an Independent Subtype of Idiopathic Multicentric Castleman Disease. Int J Mol Sci. 2022;23(18):10301. https://doi.org/10.3390/ijms231810301
41. Gao YH, Liu YT, Zhang MY, Li SY, Fajgenbaum DC, Zhang L, Li J. Idiopathic multicentric Castleman disease (iMCD)-idiopathic plasmacytic lymphadenopathy: A distinct subtype of iMCD-not otherwise specified with different clinical features and better survival. Br J Haematol. 2024;204(5):1830–37. https://doi.org/10.1111/bjh.19334. Epub 2024 Feb 15

SUPPORTING INFORMATION

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