Original Article

Hemosiderin deposition in lymph nodes of patients with plasma cell type Castleman disease

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Running title: Hemosiderin deposition in CD

Abstract

Plasma cell type Castleman disease (PCD) is a rare idiopathic atypical lymphoproliferative disorder. Differential diagnosis between PCD and IgG4-related disease (IgG4-RD) based on histology alone can be difficult. Given that PCD often presents with abundant hemosiderin deposition, lymph node lesions obtained from 22 PCD patients and 12 IgG4-RD patients were analyzed with Prussian blue staining to clarify whether hemosiderin deposition is effective in distinguishing these two diseases. This analysis disclosed that hemosiderin was more densely deposited in PCD than in IgG4-RD. The median number of Prussian blue-positive cells ± standard deviation (SD) in PCD and IgG4-RD cases was 13 ± 36 cells/3HPFs and 4 ± 8 cells/3HPFs (P = 0.034), respectively. Additionally, we analyzed the relationship between hemosiderin deposition and levels of serum interleukin (IL)-6, serum C-reactive protein (CRP), and anemia-related biomarkers. We found that hemosiderin deposition was significantly correlated with the level of serum CRP (P = 0.045); however, no significant association was observed between hemosiderin deposition and serum IL-6 levels (P = 0.204). A moderate yet nonsignificant positive correlation was observed between hemosiderin deposition and serum hemoglobin levels (P=0.09). Furthermore, no significant association was observed between hemosiderin deposition and serum iron levels (P = 0.799). In conclusion, hemosiderin deposition characteristically observed in PCD, may be related to the inflammatory aggressiveness of this disease and can be potentially used for its differential diagnosis.

Keywords: hemosiderin deposition; Plasma cell type Castleman disease; IgG4-related disease; serum IL-6; serum C-reactive protein

Introduction

Castleman disease (CD) is a rare idiopathic atypical lymphoproliferative disorder[1], with two major histological variants, designated hyaline vascular (HV-CD) and plasma cell (PCD) according to histopathological findings of affected lesions[2,3]. The typical characteristics associated with HV-CD include concentric proliferation of mantle zone lymphocytes with hyalinized vascular proliferation in germinal centers and interfollicular areas[3]. In contrast, PCD is characterized by dense mature plasma cell proliferation in expanded interfollicular areas[3]. PCD patients often present with systemic manifestations including fever with abnormal laboratory findings such as anemia, hypoalbuminemia, elevated C-reactive protein (CRP), and hypergammaglobulinemia[4]. These characteristics are believed to be caused by dysregulated overproduction of interleukin (IL)-6, which is a pleiotropic cytokine that regulates immune responses[5].

IgG4-related disease (IgG4-RD) is a recently recognized systemic syndrome characterized by mass-forming lesions and an elevated serum IgG4 level[6]. The disease involves fibrosis and severe lymphoplasmacytic infiltration with abundant IgG4-positive cells in various organs, including lymph nodes[7,8]. IgG4-related lymphadenopathy often lacks fibrosis and phlebitis, in contrast to other lesions affected in IgG4-RD[8,9]. Five histological subtypes have been described: multicentric

Castleman-like (type I), reactive follicular hyperplasia-type (type II), interfollicular expansion and immunoblastosis (type III), progressively transformed germinal centers (PTGC)-type (type IV), and inflammatory pseudotumor-like (type V)[8,10].

As PCD and IgG4-RD are both multi-organ disorders with similar histologic features of a plasma cell-rich inflammatory infiltrate, it is sometimes difficult to histologically differentiate PCD from IgG4-RD[11]. Furthermore, PCD frequently presents with variable lesions infiltrated by large numbers of IgG4-positive plasma cells, accompanied by elevated serum IgG4 levels[11,12].

Moreover, hemosiderin deposition has regularly been observed in lymph node lesions of PCD patients, hence we decided to assess the usefulness of hemosiderin deposition in differentially diagnosing PCD and IgG4-RD.

Methods and Materials

Case selection

Tissue specimens of lymph nodes were examined from 22 patients with PCD and 12 patients with IgG4-RD. All cases were retrieved from the surgical pathology consultation files of the Department of Pathology, Okayama University, Japan. The study protocol was approved by the Institutional Review Board of Okayama University, Okayama, Japan.

PCD cases with available laboratory data were examined. These cases were diagnosed based on clinical, abnormal laboratory, and pathological findings. The PCD patients consisted of 15 males and seven females aged 35 to 68 years (mean =

52.18 years). Further, 14 and seven patients presented with multiple and localized lymph node swelling, respectively. Among the 22 cases of PCD, nine inguinal, seven cervical, three axillary, one intra-abdominal, one supraclavicular and one mediastinum lymph node biopsy specimen were examined. Extra nodal lesions were also detected in 14 cases. Laboratory data are summarized in Table 1.

IgG4-RD patients diagnosed according to a previous report consisted of six males and six females aged 36 to 82 years (mean = 60.8 years)[7]. Of the 12 patients, three presented with systemic lymph node swelling and nine with localized lymph node swelling. Furthermore, six cervical, one axillary, one mediastinum, one inguinal, one submaxillary, one supraclavicular and one parotid lymph node biopsy specimens were examined. Histological subtypes of these IgG4-related lymphadenopathy samples consisted of five cases of type I, two cases of type II, and five cases of type IV. Extra nodal lesions were also detected in four cases. Laboratory data are summarized in Table 1.

Histological examination

Surgically biopsied lymph node specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial 4-μm-thick sections were cut from paraffin-embedded tissue blocks and stained with hematoxylin and eosin (HE).

Prussian blue staining

Non-stained 4-μm-thick sections were deparaffinized with 100% xylene, followed by replacement of the xylene with 100% alcohol, and finally washed with distilled water. The specimens were treated with a solution containing a mixture of potassium

ferrocyanide and hydrochloric acid for 30 minutes and washed with distilled water. They were then treated with Kernechtrot for 5 minutes and washed with distilled water. They were dehydrated with 100% alcohol, cleared with 100% xylene, and finally covered with a coverslip and water-soluble mounting medium. The number of Prussian blue-positive cells was counted in areas with the highest density of Prussian blue-positive cells in three high-powered fields (3HPFs).

Statistical analysis

Data are shown as median value ± standard deviation (SD). Comparisons between two groups were conducted by a nonparametric version of the Mann-Whitney U test. Spearman rank-based correlation coefficients were used to estimate the association between two continuous variables, and corresponding p values were also determined. All data were analyzed using SPSS software v.21.0 (SPSS Inc., Chicago, IL, USA). Results with $P < 0.05$ were regarded as significant.

Results

Hemosiderin deposition

Lymph node specimens from both PCD and the five cases of Type I IgG4-related lymphadenopathy showed atrophic to hyperplastic germinal centers and expanded interfollicular areas with sheets of plasma cells. Two case of Type II IgG4-related lymphadenopathy exhibited hyperplastic germinal centers and mature plasma cell infiltration in interfollicular areas. Five cases of Type IV IgG4-related lymphadenopathy showed follicular hyperplasia with PTGC; abundant plasma cells

were infiltrating the germinal centers, and many eosinophils were observed in the interfollicular zone. Macrophages were also observed in the interfollicular areas of PCD and IgG4-RD. Following HE staining, hemosiderin was detected in macrophages as a granular yellow-brown pigment in PCD, whereas little hemosiderin was detected in IgG4-RD (Fig. 1). Prussian blue staining clearly revealed the hemosiderin deposition. Prussian blue-positive cells were detected in interfollicular areas of PCD specimens, whereas no or very few Prussian blue-positive cells were seen in IgG4-RD cases (Fig. 1). The median number of Prussian blue-positive cells in PCD and IgG4-RD cases was 13 ± 36 cells/3HPFs (range: 0-113 cells/3HPFs) and 4 ± 8 cells/3HPFs (range: 1-27 cells/3HPFs) (Fig. 2) (P = 0.034).

Association between hemosiderin deposition and anemia-related markers

An exploratory correlation analysis was performed to examine the relationship between hemosiderin deposition and anemia-related markers such as serum iron levels and serum hemoglobin values. Serum iron levels were examined in 13 patients with PCD (range: 16-69 μg/dl). No correlation was observed between hemosiderin deposition and serum iron levels (r = -0.078, p = 0.799) (Fig. 3). The median serum hemoglobin values in PCD were 11.10 ± 1.79 (g/dl) (range: 7.1-13.2 g/dl). A moderate yet nonsignificant positive correlation was observed between serum hemoglobin levels and Prussian blue-positive cells in 3HPFs (r=0.3793, p=0.09) (Fig. 3).

Relationship between hemosiderin deposition and serum IL-6/CRP

Serum IL-6 levels were elevated in all available PCD cases (n = 16). The median

serum IL-6 levels in PCD were 16.1 ± 12.09 (pg/mL). An association was observed between serum IL-6 levels and the number of Prussian blue-positive cells in 3HPFs with no statistical significance ($r = 0.3352$, $p = 0.204$) (Fig. 3).

Correlation of serum CRP levels and hemosiderin deposition in PCD was assessed in PCD cases ($n = 21$). In all the cases, serum CRP levels exhibited levels above the reference range. The positive correlation was statistically significant between CRP levels and hemosiderin deposition in 3HPFs ($r = 0.4420$, $p = 0.045$) (Fig. 3).

Discussion

In this study, hemosiderin deposition was densely observed in PCD patient samples, while very sparse, or absent deposition was seen in IgG4-RD. It is sometimes difficult to distinguish PCD from IgG4-RD, as they have similar histological features including plasma cell infiltration in the interfollicular areas; additionally, PCD patient lymph nodes can also exhibit abundant infiltration of IgG4-positive cells[11,12]. Specific characteristics have been reported as useful in differentiating PCD from IgG4-RD, including the presence of IgA-positive cells in PCD, and eosinophilic infiltration observed in IgG4-RD[11,13]. Furthermore, hemosiderin deposition appears to be useful in differentiating between the two diseases. A useful approach to distinguish PCD from IgG4-RD may, therefore, be to evaluate a combination of factors including hemosiderin deposition, the presence of IgA-positive cells, and eosinophil infiltration. Currently, however, it is noted that the differential diagnosis between the two diseases requires comprehensive diagnostic

procedures based on not only histopathological findings but also laboratory data and clinical findings[3].

Hemosiderin is an iron-storage complex observed as a granular yellow-brown degradation product via HE staining. The deposit is tiny and often inconspicuous unless using Prussian blue staining. Hemosiderin is derived from the breakdown of hemoglobin or from an abnormal metabolic pathway of ferritin[14]. When damaged or destroyed erythrocytes leave a ruptured blood vessel they may release hemoglobin into the extracellular space. The damaged red blood cells or the released hemoglobin can then be phagocytosed by macrophages[14]. As hepcidin is known to not only inhibit intestinal iron absorption but also block iron release from macrophages[15], hemosiderin deposition can be increased when hepcidin is upregulated. Moreover, since IL-6 is known to be a key inducer of hepatic hepcidin synthesis[16], overexpression of hepcidin is likely to be associated with the hemosiderin deposition in the tissue of patients with PCD.

It has been postulated that the overproduction of IL-6 drives PCD development[5]. Although the positive relationship between hemosiderin deposition and serum IL-6 levels was not statistically significant in our study, a positive correlation between serum CRP levels and hemosiderin deposition was observed. As CRP expression is induced by IL-6 during inflammation, CRP has been considered a relevant biomarker for indirect measurement of IL-6 bioactivity[17,18]. Thus, we propose that hemosiderin deposition may be associated with PCD disease activity.

It is well documented that PCD causes microcytic anemia, mimicking iron-deficiency anemia[1]. PCD patients in our study also presented with anemia and low serum iron levels. Although PCD patients with severe anemia were speculated to have high disease activity and show abundant hemosiderin deposition, our data showed no significant relationship between hemosiderin deposition and serum iron or hemoglobin levels. This might be because anemia probably counteracts the effect of IL-6 on hepcidin production. Plasma erythropoietin (EPO) has been reported to mediate hepcidin suppression in response to augmented hematopoietic activity[19]. As the anemia worsens, EPO concentrations rise, leading to downregulation of IL-6-induced hepcidin production[20].

Conclusion

Hemosiderin was conspicuously deposited in lymph node specimens from patients with PCD. This finding may be useful in differentiating between PCD and IgG4-RD. Hepcidin may be closely related with the hemosiderin deposition in PCD. Our data indicates that PCD disease activity is positively correlated with hemosiderin deposition.

Competing interests

The authors have no competing interests to declare.

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Figure legends

Fig. 1: Histological examination and Prussian blue staining (a, c) Plasma cell type Castleman disease (PCD) (b, d) IgG4-related disease (IgG4-RD) (a, b) HE staining (200×) (c, d) Prussian blue staining (100×). (a, b) Hemosiderin deposition was observed in PCD, but not in IgG4-RD. (c) Prussian blue staining revealed hemosiderin deposition in the interfollicular area of PCD. (d) Hemosiderin deposition was not detected in IgG4-RD.

Fig. 2: Hemosiderin deposition counts in PCD and IgG4-RD cases Prussian blue-positive cells in PCD and IgG4-RD were shown in boxplots with whiskers representing minimum to maximum values. Differences between the two groups were evaluated with the Mann-Whitney U test (*$P < 0.05$). A higher number of Prussian blue-positive cells were observed in PCD than in IgG4-RD.

Fig. 3: Relationship between hemosiderin deposition and serum Fe/Hb/IL-6/ CRP in PCD An association between Prussian blue-positive cells in three high power fields (3HPF) and serum (a) iron, (b) hemoglobin, (c) IL-6, and (d) CRP. The positive correlation was significant between CRP levels and hemosiderin in 3HPFs ($P < 0.05$)