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ORIGINAL ARTICLE

Establishment of a flow cytometry assay for detecting paroxysmal nocturnal hemoglobinuria-type cells specific to patients with bone marrow failure

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Abstract

Minor populations of glycosylphosphatidylinositol-anchored protein-deficient (GPI[−]) cells in the peripheral blood may have a prognostic value in bone marrow failure (BMF). Our objective is to establish the optimal flow cytometry (FCM) assay that can discriminate GPI(−) populations specific to BMF from those of healthy individuals. To identify a cut-off that discriminates GPI(−) rare cells from GPI(+) cells, we determined a position of the borderline that separates the GPI(−) from GPI(+) cells on a scattergram by testing more than 30 healthy individuals, such that no GPI(−) dot fell into the upper left quadrant where fluorescein-labeled aerolysin (FLAER)$^{-}$CD11b^{+} granulocytes and CD55^{-}CD59^{-} glycophorin A^{+} erythrocytes were positioned. This method allowed us to define ≥ 0.003% CD11b^{+}FLAER^{-} granulocytes and ≥ 0.005% glycophorin A^{+}CD55^{-}CD59^{-} erythrocytes to be specific to BMF patients. Longitudinal cross-validation studies showed minimal (< 0.02%) inter-laboratory differences in the GPI(−) cell percentage. An analysis of 1210 patients with BMF revealed a GPI(−) cell population in 56.3% of patients with aplastic anemia and 18.5% of patients with myelodysplastic syndrome. The GPI(−) granulocyte percentages was 0.003–0.01% in 3.7% of patients. This FCM assay effectively identified an increase in the percentage of GPI(−) rare cells that are specific to BMF patients and allowed different laboratories to accurately detect 0.003–0.01% of pathological GPI(−) cells.

Keywords Flow cytometry · Bone marrow failure · PNH-type cells

Introduction

Glycosylphosphatidylinositol-anchored protein (GPI-AP)-deficient (GPI[−]) cells are known to be present in patients with bone marrow failure (BMF), such as those with acquired aplastic anemia (AA) or myelodysplastic syndromes (MDS), even when patients have no signs of hemolysis [1, 2]. Although the percentages of paroxysmal nocturnal hemoglobinuria (PNH)-type cells in subclinical PNH patients are generally low [3], even small populations of PNH-type cells could strongly influence the prognosis of patients with BMF. Their presence can predict a good response to immunosuppressive

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CORRECTION

Correction to: Effects of eculizumab treatment on quality of life in patients with paroxysmal nocturnal hemoglobinuria in Japan

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In the original publication of this article, Tables 2, 3 and 4 were published incorrectly. The corrected Tables 2, 3 and 4 are given in the following pages.

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ORIGINAL ARTICLE

Effects of eculizumab treatment on quality of life in patients with paroxysmal nocturnal hemoglobinuria in Japan

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Abstract

In paroxysmal nocturnal hemoglobinuria (PNH), various symptoms due to intravascular hemolysis exert a negative impact on patients' quality of life (QOL). To determine clinical factors related with improvements in QOL in PNH patients treated, we analyzed changes in QOL scales in PNH patients treated with eculizumab based on data collected from post-marketing surveillance in Japan. Summary statistics were obtained using figures from QOL scoring systems and laboratory values, and evaluated by *t* test. One-year administration of eculizumab improved the most QOL items in comparison with the baseline. In particular, significant improvement of EORTC QLQ-C30 was observed in fatigue, dyspnea, physical function, and global health status. Canonical correlation analysis revealed a high correlation between QOL and laboratory values. Changes in serum lactate dehydrogenase (LDH) and hemoglobin showed strong correlations with QOL improvement. Quality of life improvement was independent of patients' baseline characteristics of co-occurrence of bone marrow failure (BMF), or the degree of LDH. In this analysis, we found that the degree of QOL improvement was independent of the baseline LDH before eculizumab treatment and of co-occurrence of BMF. Paroxysmal nocturnal hemoglobinuria patients who have not received eculizumab treatment due to mild hemolysis may benefit from eculizumab treatment.

Keywords PNH · QOL · Patient-reported outcome · Complement inhibitor · Eculizumab

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REGULAR ARTICLE

blood advances

Sustained clonal hematopoiesis by HLA-lacking hematopoietic stem cells without driver mutations in aplastic anemia

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Key Points

- HSPCs that lack HLA class I alleles can sustain clonal hematopoiesis without driver mutations or telomere attrition in AA patients.
- 6pLOH may confer a survival advantage to HSPCs with age-related somatic mutations, leading to the clonal expansion of mutant HSPCs.

Clonal hematopoiesis by hematopoietic stem progenitor cells (HSPCs) that lack an HLA class I allele (HLA$^-$ HSPCs) is common in patients with acquired aplastic anemia (AA); however, it remains unknown whether the cytotoxic T lymphocyte (CTL) attack that allows for survival of HLA$^-$ HSPCs is directed at nonmutated HSPCs or HSPCs with somatic mutations or how escaped HLA$^-$ HSPC clones support sustained hematopoiesis. We investigated the presence of somatic mutations in HLA$^-$ granulocytes obtained from 15 AA patients in long-term remission (median, 13 years; range, 2-30 years). Targeted sequencing of HLA$^-$ granulocytes revealed somatic mutations (*DNMT3A*, n = 2; *TET2*, *ZRSR2*, and *CBL*, n = 1) in 3 elderly patients between 79 and 92 years of age, but not in 12 other patients aged 27 to 74 years (median, 51.5 years). The chronological and clonogenic analyses of the 3 cases revealed that *ZRSR2* mutation in 1 case, which occurred in an HLA$^-$ HSPC with a *DNMT3A* mutation, was the only mutation associated with expansion of the HSPC clone. Whole-exome sequencing of the sorted HLA$^-$ granulocytes confirmed the absence of any driver mutations in 5 patients who had a particularly large loss of heterozygosity in chromosome 6p (6pLOH) clone size. Flow–fluorescence in situ hybridization analyses of sorted HLA$^+$ and HLA$^-$ granulocytes showed no telomere attrition in HLA$^-$ granulocytes. The findings suggest that HLA$^-$ HSPC clones that escape CTL attack are essentially free from somatic mutations related to myeloid malignancies and are able to support long-term clonal hematopoiesis without developing driver mutations in AA patients unless HLA loss occurs in HSPCs with somatic mutations.

Introduction

Acquired aplastic anemia (AA) is an immune-mediated bone marrow failure triggered by T lymphocytes specific to hematopoietic stem progenitor cells (HSPCs). Approximately 70% of patients respond to immunosuppressive therapy (IST) and show the sustained restoration of hematopoiesis.[1-4] However, 5% to 10% of AA patients develop myelodysplastic syndromes (MDSs) or acute myelogenous leukemia (AML) after a long latency period, and AA is therefore regarded as a preleukemic state similar to lower-risk MDSs. In keeping with this concept, various studies have revealed clonal hematopoiesis in a subset of AA patients. Studies using the lyonization of genes on the X chromosome have suggested the presence of clonal hematopoiesis in ~30% of female patients with AA.[5,6] Current studies using

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*T.I. and T.K. equally contributed to this work.

The nucleotide sequence data reported in this article are available in the DDBJ Japanese Genotype-phenotype Archive for genetic and phenotypic human data (accession number JGAS00000000094).

The full-text version of this article contains a data supplement.

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CORRESPONDENCE

Escape hematopoiesis by donor-derived 6pLOH(+) hematopoietic stem cells in a marrow transplant recipient with late graft failure

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Late graft failure (LGF) is a rare but serious complication in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients with complete donor chimerism. A second allo-HSCT from the original donor with or without preconditioning may be effective [1, 2]; however, it is often associated with transplant-related mortality. Some patients with LGF can be successfully treated with immunosuppressive therapy to inhibit donor T cells capable of specifically eliminating autologous hematopoietic stem cells [3, 4]. However, it is difficult to confirm the involvement of donor-derived cytotoxic T cells in the development of LGF. Here, we report a case of donor-type LGF, which occurred 17 years after an allogeneic bone marrow transplantation from a 6/8 human leukocyte antigen (HLA)-matched half-sibling donor. Immune-mediated hematopoietic failure was diagnosed based on the presence of HLA-A allele-lacking leukocytes (HLA-LLs) attributed to copy number neutral loss of heterozygosity in the short arm of chromosome 6 (6pLOH).

A 38-year-old female was diagnosed with having severe aplastic anemia in November 1997. She did not respond to treatment with horse anti-thymocyte globulin (ATG), cyclosporine (CsA), and danazol: thus, there was a risk of death due to severe pancytopenia. As no HLA-matched donors were available from her family and the Japan Marrow Donor Program, she underwent bone marrow transplantation from an HLA-haploidentical donor at the age of 40 after a myeloablative conditioning regimen with cyclophosphamide (60 mg/kg/day on days −4 and −3), TBI (3 Gy × 2/day on days −9 and −8), and cytarabine (3 g/m^2 × 2/day on days −6 and −5). The HLA haplotypes of the donor were *A*02:01-B*54:01-C*01:02-DRB1*15:02/A*26:01-B*40:02-C*03:04-DRB1*09:01* and the HLA haplotypes of the patient were A2/A24-B54/B61-Cw1/Cw9-DRB1*15:02/DRB1*09:01, which were estimated to be *A*02:01-B*54:01-C*01:02-DRB1*15:02/A*24:02-B*40:02-C*03:03-DRB1*09:01* based on the HLA frequency in the Japanese population. Neutrophil engraftment occurred on day 19, and transfusion independence was achieved on day 91 after bone marrow transplantation. She developed chronic graft-versus-host disease (cGVHD) of the skin, liver, and lungs on day 366 and required long-term treatment with CsA and prednisolone until August 2010. When CsA was discontinued at the age of 51, her white blood cell count was 9.4×10^9/L, hemoglobin level was 12.2 g/dL, and platelet count was 92×10^9/L. Six years later, pancytopenia developed without any signs of infection and recurrence of cGVHD. The complete blood counts were as follows: white blood cells 2.9×10^9/L with 78% neutrophils and 7.5% lymphocytes, hemoglobin level 9.6 g/dL, platelets 12×10^9/L, and reticulocytes 65×10^9/L. Bone marrow examination showed hypocellularity without abnormal cells and dysplastic signs. Sex chromosome analyses of her peripheral blood granulocytes and T cells showed complete donor chimerism. LGF with complete donor chimerism was diagnosed. A high-sensitivity flow cytometry assay revealed glycosylphosphatidyl-inositol-anchored protein (GPI-AP)-deficiency in 0.082% granulocytes and 0.006% erythrocytes (Fig. 1a), suggesting that the patient's LGF was immune-mediated [3].

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Experimental Hematology

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Disease modeling of bone marrow failure syndromes using iPSC-derived hematopoietic stem progenitor cells

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The plasticity of induced pluripotent stem cells (iPSCs) with the potential to differentiate into virtually any type of cells and the feasibility of generating hematopoietic stem progenitor cells (HSPCs) from patient-derived iPSCs (iPSC-HSPCs) has many potential applications in hematology. For example, iPSC-HSPCs are being used for leukemogenesis studies and their application in various cell replacement therapies is being evaluated. The use of iPSC-HSPCs can now provide an invaluable resource for the study of diseases associated with the destruction of HSPCs, such as bone marrow failure syndromes (BMFSs). Recent studies have shown that generating iPSC-HSPCs from patients with acquired aplastic anemia and other BMFSs is not only feasible, but is also a powerful tool for understanding the pathogenesis of these disorders. In this article, we highlight recent advances in the application of iPSCs for disease modeling of BMFSs and discuss the discoveries of these studies that provide new insights in the pathophysiology of these conditions. © 2019 Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Aplastic anemia (AA) is a life-threatening bone marrow failure (BMF) disorder, resulting in bone marrow hypoplasia, infection and hemorrhage, and severe peripheral pancytopenia. Although the most cases of AA are acquired and associated with the autoimmune destruction of hematopoietic stem progenitor cells (HSPCs) in the BM, in some cases, the BMF is caused by genetic or inherited anomalies that impair hematopoiesis [1]. The destruction or dysfunction of HSPCs in the BM of patients with BMF syndromes (BMFSs) limits the study of these disorders because the use of conventional in vitro HSPC culture or in vivo animal models for creating patient-specific disease modeling is technically impossible due to the unavailability of patient-derived HSPCs. With the development of induced pluripotent stem cells (iPSCs) [2], a promising venue for the study of rare diseases such as BMFSs has been opened. The generation of patient-derived iPSCs and their subsequent differentiation into iPSC-HSPCs offer a unique opportunity for generating disease models to study several genetic and immune backgrounds of BMFSs, facilitating the investigation of human rare diseases based on individual patients' phenotypes (Figure 1). Previously, we summarized some aspects of using iPSCs for understanding AA pathogenesis and the methods of establishing animal models for acquired AA (aAA) using iPSCs [3]. To achieve the goal of this review, we will focus on the previous successful trials to generate iPSCs from patients with different BMFSs. By drawing upon the broad experimental expertise of the previous published works, we will try to summarize the possible future application of this technology in understanding the pathogenesis of BMFSs and the potential challenges encountered using iPSC-based models of these disorders.

Generation of iPSC clones from patients with inherited BMFSs

Inherited BMFSs are a rare group of disorders often developing in childhood that are characterized by BMF with a marked reduction of all hematopoietic lineages or a single-cell lineage usually in association with one or more physical abnormalities [4]. Although the genetic lesions linked with most inherited BMFSs have been identified, some of the cellular events resulting from such genetic aberrations have not been clarified [5]. The utilization of iPSC-derived hematopoietic cells

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REGULAR ARTICLE

blood advances

Frequent *STAT3* mutations in CD8$^+$ T cells from patients with pure red cell aplasia

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Key Points

- Somatic *STAT3* mutations were frequently found in PRCA with or without T-LGLL.
- *STAT3* mutation–positive PRCA patients were less responsive to cyclosporine treatment than mutation-negative patients.

Dysregulation of T-cell–mediated immunity is responsible for acquired pure red cell aplasia (PRCA). Although *STAT3* mutations are frequently detected in patients with T-cell large granular lymphocytic leukemia (T-LGLL), which is often complicated by PRCA and which is also reported to be associated with acquired aplastic anemia (AA) and myelodysplastic syndrome (MDS), whether *STAT3*-mutated T cells are involved in the pathophysiology of PRCA and other types of bone marrow failure remains unknown. We performed *STAT3* mutation analyses of the peripheral blood mononuclear cells from PRCA patients (n = 42), AA (n = 54), AA–paroxysmal nocturnal hemoglobinuria (AA-PNH; n = 7), and MDS (n = 21) using an allele-specific polymerase chain reaction and amplicon sequencing. *STAT3* mutations were not detected in any of the 82 patients with AA/PNH/MDS but were detected in 43% of the 42 PRCA patients. In all 7 *STAT3*-mutation–positive patients who were studied, the *STAT3* mutations were restricted to sorted CD8$^+$ T cells. The prevalence of *STAT3* mutation in idiopathic, thymoma-associated, autoimmune disorder–associated, and T-LGLL–associated PRCA was 33% (5 of 15), 29% (2 of 7), 20% (1 of 5), and 77% (10 of 13), respectively. The *STAT3*-mutation–positive patients were younger (median age, 63 vs 73 years; $P = .026$) and less responsive to cyclosporine (46% [6 of 13] vs 100% [8 of 8]; $P = .0092$) in comparison with *STAT3*-mutation–negative patients. The data suggest that *STAT3*-mutated CD8$^+$ T cells may be closely involved in the selective inhibition of erythroid progenitors in PRCA patients.

Introduction

Somatic mutations of *STAT3*, one of the STAT-signaling molecules, are among the most frequent types of genetic alterations in patients with T- or natural killer (NK)–cell lymphoma, especially patients with T-cell large granular lymphocytic leukemia (T-LGLL),[1] chronic lymphoproliferative disorders of NK cells (CLPD-NK),[2] aggressive NK-cell leukemia,[3] and chronic adult T-cell leukemia/lymphoma (ATL/L).[4] Activating mutations in the Src-homology 2 (SH-2) domain of *STAT3* that led to the constitutional phosphorylation of STAT3 and enhanced transcriptional activity by the JAK/STAT-signaling pathways are considered to be mechanisms of aberrant T-/NK-cell proliferation, based on the *STAT3* mutations in these lymphoid malignancies.[1,2]

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ORIGINAL ARTICLE

Efficacy and safety of switching to nilotinib in patients with CML-CP in major molecular response to imatinib: results of a multicenter phase II trial (NILSw trial)

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Abstract

We evaluated the efficacy and safety of switching to nilotinib in CML-CP patients who had achieved MMR with continuous detectable BCR-ABL1 transcript levels after long-term imatinib treatment. Patients who had achieved MMR, but not deep molecular response (DMR), after > 18 months from the initiation of imatinib received nilotinib 400 mg twice daily for up to 24 months. BCR-ABL1 transcript levels were assessed every 3 months. Thirty-eight patients with a median age of 57.5 years (range 22–76 years) were evaluated. Twenty-seven patients completed 24 months of nilotinib treatment; 11 discontinued nilotinib due to retraction of consent (three patients), loss of MMR (1), intolerance (3) or AEs (5). Twenty patients [52.6%, (90% CI 38.2–66.7%)] achieved DMR. The cumulative incidence of achieving DMR by the time of 3, 6, 9, 12, 15, 18, and 21 months was 22.9, 37.7, 47.0, 53.7, 53.7, 53.7, and 53.7%, respectively. Adverse events were consistent with those reported in other nilotinib studies. Patients experienced each of the following cardiovascular complications: atrial fibrillation (G2), chest tightness and dyspnea (G1), myocardial infarction (G2) and heart failure (G3) ($n = 1$ for each complication). This study indicates nilotinib achieves strong, rapid induction of DMR for patients who achieved MMR after long-term imatinib therapy.

Keywords Chronic myelogeneous leukemia · Deep molecular response · Major molecular response · Imatinib · Nilotinib

Introduction

Imatinib has dramatically improved the prognosis in patients with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia in chronic phase (CML-CP) [1–3]. Today, the goals of treatment for CML-CP with tyrosine kinase inhibitors (TKIs) are mainly evaluated on monitoring the BCR-ABL1 transcript level with real-time quantitative polymerase chain reaction (RQ-PCR) from peripheral blood [4, 5]. International Randomized Study of Interferon versus

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Genome-wide CRISPR-Cas9 screen identifies leukemia-specific dependence on a pre-mRNA metabolic pathway regulated by DCPS

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T.Y., M.C.C., L.P., D.E.B. and T.M. designed CRISPR-Cas9 screen experiments. T.Y., Y.S. and T.M reviewed CRISPR screen data. T.M., M.M., Y.I., Y.S. and M.N. executed CRISPR-CAS9 experiments, cell biology experiments, RNA-Seq, western blot analysis, immunohistochemistry and in vivo mouse studies, supervised by F.A., L.P., S.H.O., K.A., D.E.B. and T.M. T.M. performed mass-spectrometry and analyzed proteomic data. M.S., S.B. Y.S., Q.Y. and L.P. analyzed RNA-Seq data. M.S., M. A. R. and B.R. provided patient data. M.C.C., V.A.C.S., M.A.C. and C.M.T. analyzed CRISPR saturation mutagenesis data, supervised by L.P. and D.E.B. T.Y and T.M. wrote the manuscript with help from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Research paper

Differing clinical features between Japanese and Caucasian patients with myelodysplastic syndromes: Analysis from the International Working Group for Prognosis of MDS

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ABSTRACT

Clinical features of myelodysplastic syndromes (MDS) could be influenced by many factors, such as disease intrinsic factors (e.g., morphologic, cytogenetic, molecular), extrinsic factors (e.g, management, environment), and ethnicity. Several previous studies have suggested such differences between Asian and European/USA countries. In this study, to elucidate potential differences in primary untreated MDS between Japanese (JPN) and Caucasians (CAUC), we analyzed the data from a large international database collected by the International

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Prognostic Impact of Donor Source on Allogeneic Hematopoietic Stem Cell Transplantation Outcomes in Adults with Chronic Myelomonocytic Leukemia: A Nationwide Retrospective Analysis in Japan

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative therapeutic option for patients with chronic myelomonocytic leukemia (CMML). We retrospectively compared the post-transplantation outcomes of 159 patients with CMML who underwent allo-HSCT using 4 types of donor sources: HLA-matched related donor graft, unrelated bone marrow (U-BM), unrelated cord blood (U-CB), and HLA-mismatched related donor graft. The median patient age at allo-HSCT was 54 years (range, 16 to 75 years). In multivariate analyses, the use of HLA-matched related donor grafts correlated with better overall survival than U-BM (hazard ratio [HR], 2.05; 95% confidence interval [CI], 1.21 to 3.48; $P = .008$), U-CB (HR, 3.80; 95% CI, 2.07 to 6.95; $P < .001$), or HLA-mismatched related donor grafts (HR, 6.18; 95% CI, 2.70 to 14.15; $P < .001$). Mortality after the relapse or progression of CMML did not significantly differ among the 4 types of donor source. Transplantation-related mortality was highest in recipients of U-CB (HR, 3.32; 95% CI, 1.33 to 8.26; $P = .010$). In patients with CMML, allo-HSCT using an alternative donor may contribute to durable remission; however, further improvements in transplantation-related mortality are required for this type of transplantation.

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Research paper

Interobserver concordance of assessments of dysplasia and blast counts for the diagnosis of patients with cytopenia: From the Japanese central review study

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ABSTRACT

The diagnosis of myelodysplastic syndromes (MDS) is based on morphology and cytogenetics. However, limited information is currently available on the interobserver concordance of the assessment of dysplastic lineages (< 10% or ≥10% in bone marrow (BM)). The revised International Prognostic Scoring System (IPSS-R) described a new threshold (2%) for BM blasts. However, the interobserver concordance of the categories (0–≤2% and > 2– < 5%) has limited data. The purpose of the present study was to investigate the assessment of dysplastic lineages and IPSS-R reproducibility. Our study was divided into two Steps. In each Step, the microscopic examinations were performed separately by two morphologists. Regarding the category of BM blasts ≤2% and > 2– < 5%, interobserver agreement was more than 'moderate' in all pairs (kappa test: 0.43–0.90). Regarding dysgranulopoiesis (dysG) and dyserythropoiesis (dysE) in BM, interobserver agreement was more than 'moderate' in all pairs (kappa test, dysG: 0.45–0.96, dysE: 0.45–0.81). Regarding the category of dysmegakaryopoiesis (dysMgk) in BM, interobserver agreement was more than moderate in 4 out of 5 pairs (kappa test: 0.58–1.00), and was fair for one pair (kappa test: 0.37). We consider that high interobserver concordance may be possible for the BM blast cell count (≤2% or > 2– < 5%) and dysplasia (< 10% or ≥10%) of each lineage.

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ARTICLE

Acute myeloid leukemia

Molecular pathogenesis of disease progression in *MLL*-rearranged AML

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Abstract

Leukemic relapse is frequently accompanied by progressively aggressive clinical course. To understand the molecular mechanism of leukemic relapse, *MLL/AF9*-transformed mouse leukemia cells were serially transplanted in C57BL/6 mice ($N = 96$) by mimicking repeated recurrences, where mutations were monitored by exome sequencing ($N = 42$). The onset of leukemia was progressively promoted with advanced transplants, during which increasing numbers of somatic mutations were acquired ($P < 0.005$). Among these, mutations in *Ptpn11* (p.G60R) and *Braf* (p.V637E) corresponded to those identified in human *MLL*-AML, while recurrent mutations affecting *Msn* (p.R295C) were observed only in mouse but not in human *MLL*-AML. Another mutated gene of interest was *Gnb2* which was reported to be recurrently mutated in various hematological neoplasms. *Gnb2* mutations (p.G77R) were significantly increased in clone size ($P = 0.007$) and associated with earlier leukemia onset ($P = 0.011$). *GNB2* transcripts were significantly upregulated in human *MLL*-AML compared to *MLL*-negative AML ($P < 0.05$), which was supported by significantly increased *Gnb2* transcript induced by *MLL/AF9* overexpression ($P < 0.001$). In in vivo model, both mutation and overexpression of *GNB2* caused leukemogenesis, and downregulation of *GNB2* expression reduced proliferative potential and survival benefit, suggesting a driver role of *GNB2*. In conclusion, alterations of driver genes over time may play an important role in the progression of *MLL*-AML.

Introduction

Despite advanced therapeutics, many leukemia patients become refractory to additional therapy, accounting for a major cause of leukemic deaths. Among major human acute myeloid leukemias (AMLs), *MLL*-rearranged AML (*MLL*-AML) is characteristic of poor prognosis due to

These authors contributed equally: Shinichi Kotani, Akinori Yoda, Ayana Kon and Keisuke Kataoka

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ARTICLE

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Integrative genomic analysis of adult mixed phenotype acute leukemia delineates lineage associated molecular subtypes

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Mixed phenotype acute leukemia (MPAL) is a rare subtype of acute leukemia characterized by leukemic blasts presenting myeloid and lymphoid markers. Here we report data from integrated genomic analysis on 31 MPAL samples and compare molecular profiling with that from acute myeloid leukemia (AML), B cell acute lymphoblastic leukemia (B-ALL), and T cell acute lymphoblastic leukemia (T-ALL). Consistent with the mixed immunophenotype, both AML-type and ALL-type mutations are detected in MPAL. Myeloid-B and myeloid-T MPAL show distinct mutation and methylation signatures that are associated with differences in lineage-commitment gene expressions. Genome-wide methylation comparison among MPAL, AML, B-ALL, and T-ALL sub-classifies MPAL into AML-type and ALL-type MPAL, which is associated with better clinical response when lineage-matched therapy is given. These results elucidate the genetic and epigenetic heterogeneity of MPAL and its genetic distinction from AML, B-ALL, and T-ALL and further provide proof of concept for a molecularly guided precision therapy approach in MPAL.

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ORIGINAL ARTICLE

PAS positivity of erythroid precursor cells is associated with a poor prognosis in newly diagnosed myelodysplastic syndrome patients

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Abstract

Myelodysplastic syndrome (MDS) is a group of clonal stem cell disorders characterized by hematopoietic insufficiency. The accurate risk stratification of patients with MDS is essential for selection of appropriate therapies. We herein conducted a retrospective cohort study to examine the prognostic value of periodic acid-Schiff (PAS) reaction-positive erythroblasts in MDS patients. We examined the PAS positivity of the bone marrow erythroblasts of 144 patients newly diagnosed with MDS; 26 (18.1%) of them had PAS-positive erythroblasts, whereas 118 (81.9%) did not. The PAS-positive group showed significantly poorer karyotypes as defined in the revised International Prognostic Scoring System (IPSS-R) and higher scores in age-adjusted IPSS-R (IPSS-RA) than the PAS-negative group. Overall survival (OS) and leukemia-free survival (LFS) were also significantly shorter in the PAS-positive group than in the PAS-negative group. Similar results were obtained when only high- and very high risk groups were analyzed using IPSS-RA. This retrospective study suggested that the PAS positivity of erythroblasts is an additional prognostic factor combined with other risk scores for OS and LFS in MDS, and our results may contribute to improved clinical decision-making and rapid risk stratification.

Keywords PAS-positive erythroblasts · Myelodysplastic syndrome · Prognosis · International Prognostic Scoring System · Revised International Prognostic Scoring System

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal stem cell disorders characterized by peripheral cytopenia and dysplastic changes in bone marrow cells and is associated with a high risk of transformation to acute myeloid leukemia (AML) [1]. The annual incidence of MDS is 3–5/100,000, with age-specific rates increasing to > 20/100,000 among individuals older than 70 years of age [2]. It is important to assess the disease risk at diagnosis using established prognostic scoring systems in order to estimate prognoses and make decisions including whether aggressive treatments, such as chemotherapy and potentially curative allogenic hematopoietic cell transplantation, are needed [3, 4]. To date, various risk assessment systems have been proposed, among which the first widely adopted model was the International Prognostic Scoring System (IPSS). In IPSS, cytogenetic subgroups, marrow blast percentages, and the extent of cytopenia are incorporated into assessments of disease risk in primary untreated MDS patients [5]. The revised IPSS

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ARTICLE

Prognostic factors of Erdheim–Chester disease: a nationwide survey in Japan

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ABSTRACT

Erdheim–Chester disease is a rare histiocytosis with insufficient clinical data. To clarify the clinical features and prognostic factors of Erdheim–Chester disease, we conducted a nationwide survey to collect the detailed data of 44 patients with Erdheim–Chester disease in Japan. The median age of onset of the participants was 51 (range: 23–76) years, and the median number of involved organs per patient was 4 (range: 1–11). The existence of central nervous system disease was correlated with older age (P=0.033), the presence of cardiovascular lesions (P=0.015), and an increased number of involved organs (P=0.0042). The median survival from the onset was 10.4 years, and >3.0 mg/dL C-reactive protein level at onset was associated with worse outcome (median survival, 14.6 *vs.* 7.4 years; P=0.0016). In a multivariate analysis, age >60 years (hazard ratio, 25.9; 95% confidence interval, 2.82–237; P=0.0040) and the presence of digestive organ involvement (hazard ratio, 4.74; 95% confidence interval, 1.05–21.4; P=0.043) were correlated with worse survival. Fourteen patients had available histological samples of Erdheim–Chester disease lesions. *BRAF*V600E mutation was detected in 11 patients (78%) by Sanger sequencing. A correlation between *BRAF* mutation status and clinical factors was not observed. Our study revealed that age and digestive organ involvement influence the outcome of Erdheim–Chester disease patients, and an inflammatory marker, such as C-reactive protein, might reflect the activity of this inflammatory myeloid neoplasm.

Introduction

Erdheim–Chester disease (ECD) is a rare non-Langerhans histiocytosis that was first reported by Jakob Erdheim and William Chester in 1930.[1] The number of reports has drastically increased recently, perhaps due to the increased recognition of the disease, and approximately 650–1000 cases have been reported.[2-4] ECD typically develops among middle-aged males, and bilateral cortical osteosclerosis occurs in more than 95% of ECD patients.[5] Furthermore, some patients experience involvements of the central nervous system (CNS), cardiovascular system, and various other organs.[6,7]

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ORIGINAL ARTICLE

Comparison of blast percentage calculated based on bone marrow all nucleated cells and non-erythroid cells in myelodysplastic syndromes with erythroid hyperplasia

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Abstract

It is controversial whether blast percentage based on all nucleated cells (ANC) or non-erythroid cells (NEC) more accurately reflects the prognosis of patients with myelodysplastic syndromes (MDS). We considered that the impact of blast percentage on survival should be similar in MDS with erythroid hyperplasia (MDS-E) and MDS with no erythroid hyperplasia (MDS-NE), and from this perspective, we retrospectively analyzed 322 patients, including 44 with MDS-E and 278 with MDS-NE. Overall survival was similar between the MDS-E and MDS-NE groups ($P = 0.94$). In a subgroup of patients with bone marrow (BM) blasts of < 5%, no difference in survival was found between MDS-E and MDS-NE by either calculation method. However, in patients with a blast percentage between 5 and 10%, a significant difference in survival was observed only when the blast percentage in MDS-E was calculated from ANC ($P < 0.001$ by ANC and $P = 0.66$ by NEC). A similar result was observed when we analyzed the remaining patients with higher blasts together with those with blasts between 5 and 10%. These results suggest that the calculation of the BM blast percentage based on NEC in MDS-E provides a blast percentage value with a clinical impact consistent with that in MDS-NE.

Keywords Myelodysplastic syndromes (MDS) · Erythroid hyperplasia · Non-erythroid cells (NEC) · Acute myeloid leukemia (AML)

Introduction

Myelodysplastic syndromes (MDS) are heterogeneous hematopoietic stem cell disorders characterized by ineffective hematopoiesis resulting in cytopenia and the risk of progression to acute myeloid leukemia (AML) [1, 2]. Treatment strategies for MDS are usually decided upon based on a prognostic scoring system that includes the percentage of bone marrow (BM) blasts, genetic abnormalities, and peripheral cytopenia [3–6]. Therefore, the accurate estimation of BM blasts is important.

In the new World Health Organization (WHO) 2016 classification, the bone marrow blast percentage in MDS is calculated based on all nucleated cells (ANC) regardless of the percentage of erythroid cells. However, some recent reports have suggested that calculation of the blast percentage based on non-erythroid cells (NEC) more accurately reflects the prognosis of MDS with erythroid hyperplasia (MDS-E) than that based on ANC [7, 8]. Thus, the method for calculating BM blasts is still controversial.

We considered that the impact of blast percentage on prognosis should be similar in MDS-E and MDS with no erythroid hyperplasia (MDS-NE). From this perspective, in this study, we classified patients according to the

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ORIGINAL RESEARCH ARTICLE

WILEY

Induction chemotherapy followed by allogeneic HCT versus upfront allogeneic HCT for advanced myelodysplastic syndrome: A propensity score matched analysis

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Abstract

To reduce post-transplant relapse, acute myeloid leukemia (AML) type remission induction chemotherapy has been attempted to reduce disease burden before allogeneic hematopoietic cell transplantation (HCT) in patients with advanced myelodysplastic syndrome (MDS). However, the efficacy of induction chemotherapy before HCT is unclear. We retrospectively analyzed the Japanese registration data of 605 adult patients, who had received allogeneic HCT for advanced MDS between 2001 and 2016, to compare the post-transplant relapse between patients who received induction chemotherapy followed by allogeneic HCT and those who received upfront HCT. Propensity score matching identified 230 patients from each cohort. There were no significant differences in overall survival and non-relapse mortality between the two groups. The cumulative incidence of relapse was significantly higher in patients who received induction chemotherapy than those who received upfront HCT. In the subgroup analyses, upfront HCT had a significantly reduced relapse incidence among patients with poor cytogenetics, those with higher international prognostic scoring system at diagnosis, and those who received reduced-intensity conditioning. Our results suggested that AML type remission induction chemotherapy before HCT did not improve post-transplant relapse and survival for adult patients with advanced MDS. Upfront HCT is preferable for patients with a poor karyotype.

KEYWORDS

myelodysplastic syndrome, allogeneic hematopoietic cell transplantation, induction chemotherapy, cytoreductive treatment, relapse, propensity score matched analysis

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ORIGINAL ARTICLE

Whole-exome analysis to detect congenital hemolytic anemia mimicking congenital dyserythropoietic anemia

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Abstract

Congenital dyserythropoietic anemia (CDA) is a heterogeneous group of rare congenital disorders characterized by ineffective erythropoiesis and dysplastic changes in erythroblasts. Diagnosis of CDA is based primarily on the morphology of bone marrow erythroblasts; however, genetic tests have recently become more important. Here, we performed genetic analysis of 10 Japanese patients who had been diagnosed with CDA based on laboratory findings and morphological characteristics. We examined 10 CDA patients via central review of bone marrow morphology and genetic analysis for congenital bone marrow failure syndromes. Sanger sequencing for *CDAN1, SEC23B*, and *KLF1* was performed for all patients. We performed whole-exome sequencing in patients without mutation in these genes. Three patients carried pathogenic *CDAN1* mutations, whereas no *SEC23B* mutations were identified in our cohort. WES unexpectedly identified gene mutations known to cause congenital hemolytic anemia in two patients: canonical *G6PD* p.Val394Leu mutation and *SPTA1* p.Arg28His mutation. Comprehensive genetic analysis is warranted for more effective diagnosis of patients with suspected CDA.

Keywords Congenital dyserythropoietic anemia · Congenital hemolytic anemia · Whole-exome analysis

Introduction

Congenital dyserythropoietic anemia (CDA) is a heterogenous group of rare congenital disorders characterized by ineffective erythropoiesis and dysplastic changes in erythroblasts. CDA is originally classified into three major types on the basis of morphological findings [1], and most patients exhibit CDA type I (CDA I) or CDA type II (CDA II). CDA has mainly been reported in Western European and Middle-Eastern countries and very few cases have been published from East Asian countries [2–4]. The clinical diagnosis of CDA is primarily based on the laboratory findings of ineffective erythropoiesis and the characteristic morphology of erythroblasts in the bone marrow (BM) [5]. Specific diagnostic tests, including the acid serum lysis test using ABO-compatible sera or the abnormality of bands 3 and 4.5 shown using sodium dodecyl sulfate polyacrylamide gel electrophoresis, are not available except in specialized diagnostic centers for CDA. However, 80–90% of patients with CDA I or CDA II carry causative mutations in *CDAN1* or *SEC23B*, respectively [6], which have increased the significance of genetic tests in the diagnosis of CDA. In the present study,

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ARTICLE

Myelodysplastic syndrome

A germline *HLTF* mutation in familial MDS induces DNA damage accumulation through impaired PCNA polyubiquitination

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Abstract

Although several causal genes of familial myelodysplastic syndromes (MDS) have been identified, the genetic landscape and the molecular pathogenesis are not totally understood. To explore novel driver genes and their pathogenetic significance, we performed whole-exome sequence analysis of four individuals from a familial MDS pedigree and 10 candidate single-nucleotide variants (*C9orf43, CYP7B1, EFHB, ENTPD7, FAM160B2, HELZ2, HLTF, INPP5J, ITPKB,* and *RYK*) were identified. Knockdown screening revealed that *Hltf* downregulation enhanced colony-forming capacity of primary murine bone marrow (BM) stem/progenitor cells. γH2AX immunofluorescent staining assay revealed increased DNA damage in a human acute myeloid leukemia (AML) cell line ectopically expressing HLTF E259K, which was not observed in cells expressing wild-type HLTF. Silencing of *HLTF* in human AML cells also led to DNA damage, indicating that *HLTF* E259K is a loss-of-function mutation. Molecularly, we found that an E259K mutation reduced the binding capacity of HLTF with ubiquitin-conjugating enzymes, methanesulfonate sensitive 2 and ubiquitin-conjugating enzyme E2N, resulting in impaired polyubiquitination of proliferating cell nuclear antigen (PCNA) in *HLTF* E259K-transduced cells. In summary, our results indicate that a familial MDS-associated *HLTF* E259K germline mutation induces accumulation of DNA double-strand breaks, possibly through impaired PCNA polyubiquitination.

Introduction

The myelodysplastic syndromes (MDS) are a group of clonal disorders of hematopoietic stem cells, characterized by bone marrow (BM) dysplasia and ineffective hematopoiesis [1, 2]. Previous comprehensive mutational analyses uncovered the genetic landscape of sporadic MDS, including frequent alterations in *Tet methylcytosine dioxygenase 2* (*TET2*), *splicing factor 3b subunit 1* (*SF3B1*), *additional sex combs like 1, transcriptional*

These authors contributed equally: Kensuke Takaoka, Masahito Kawazu

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Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Utility of nintedanib for severe idiopathic pulmonary fibrosis: a single-center retrospective study

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

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Introduction: The INPULSIS-ON trial demonstrated that nintedanib reduced decline in forced vital capacity (FVC) and low pulmonary function (%FVC < 50%) of patients with idiopathic pulmonary fibrosis (IPF). However, there is no sufficient evidence in real world.

Objectives: Reveal the utility and adverse events of nintedanib for severe IPF patients.

Methods: This was a single-center retrospective study. Patients who met the eligibility criteria of the INPULSIS trial (%FVC ≥ 50%; $\%D_{LCO}$ [diffusing capacity of the lung carbon monoxide % predicted] ≥ 30%) were classified as Mild to Moderate Group (n = 34); patients who did not meet the criteria were classified as Severe Group (n=17).

Results: The body mass index (24.7 ± 3.4 vs 22.4 ± 3.6 kg/m^2; $P = 0.021$) were significantly low in Severe Group. Main adverse events (diarrhea, nausea, liver disorder, and acute exacerbation) tended to be more in Severe Group than in Mild to Moderate Group; however, the difference was not significant ($P = 0.76$, 0.14, 0.18, and 0.67, respectively). The continuation rates over 12 months tended to be higher in Mild to Moderate Group than in Severe Group (77% vs 44%; $P = 0.027$). Log-rank test revealed that the prognosis was significantly better in Mild to Moderate Group than in Severe Group ($P = 0.014$). In the Severe Group, patients who were able to continue nintedanib for more than 3 months had significantly better prognosis compared to those who could not ($P = 0.007$).

Conclusion: The benefit from nintedanib was reduced in patients in Severe Group when compared to those in Mild to Moderate Group; however, the prognosis is expected to improve with control of side effects and long-term administration. It is more important to control the side effects in Severe Group.

Keywords: idiopathic pulmonary fibrosis, nintedanib, triple tyrosine kinase inhibitor, INPULSIS trials, forced vital capacity

Introduction

IPF is a chronic progressive interstitial pneumonia.[1] It has been reported that an average survival time of IPF is 2–3 years after diagnosis, and the prognosis of IPF is as poor as many types of cancers.[2–4] The pulmonary function test is important for evaluation of the severity of IPF. The FVC and D_{LCO} have also been reported to be important prognostic factors of IPF patients.[2,5–7]

Nintedanib is a tyrosine-kinase inhibitor and targets vascular endothelial growth factor receptors, fibroblast growth factor receptors, and platelet-derived growth factor receptors.[8,9] In the most recent international clinical practice guidelines, nintedanib received a conditional recommendation for the treatment of IPF.[10] The TOMORROW trial (nintedanib Phase II clinical trial)[11] and INPULSIS-1/2 trial

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REVIEW ARTICLE

WILEY Cancer Science

Langerhans cell histiocytosis in adults: Advances in pathophysiology and treatment

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Langerhans cell histiocytosis (LCH) is a rare systemic disorder characterized by the accumulation of CD1a+/Langerin+ LCH cells and wide-ranging organ involvement. Langerhans cell histiocytosis was formerly referred to as histiocytosis X, until it was renamed in 1987. Langerhans cell histiocytosis β was named for its morphological similarity to skin Langerhans cells. Studies have shown that LCH cells originate from myeloid dendritic cells rather than skin Langerhans cells. There has been significant debate regarding whether LCH should be defined as an immune disorder or a neoplasm. A breakthrough in understanding the pathogenesis of LCH occurred in 2010 when a gain-of-function mutation in *BRAF* (V600E) was identified in more than half of LCH patient samples. Studies have since reported that 100% of LCH cases show ERK phosphorylation, indicating that LCH is likely to be a clonally expanding myeloid neoplasm. Langerhans cell histiocytosis is now defined as an inflammatory myeloid neoplasm in the revised 2016 Histiocyte Society classification. Randomized trials and novel approaches have led to improved outcomes for pediatric patients, but no well-defined treatments for adult patients have been developed to date. Although LCH is not fatal in all cases, delayed diagnosis or treatment can result in serious impairment of organ function and decreased quality of life. This study summarizes recent advances in the pathophysiology and treatment of adult LCH, to raise awareness of this "orphan disease".

KEYWORDS
adult, histiocytosis, Langerhans cell, mitogen-activated protein kinase, proto-oncogene protein BRAF

1 | OVERVIEW OF LANGERHANS CELL HISTIOCYTOSIS

The Langerhans cell was first described by Paul Langerhans Jr. in 1868.[1] The first definitive case of Langerhans cell histiocytosis (LCH) was reported in 1893 by Alfred Hand.[2] The patient was a 3-year-old boy with polyuria, exophthalmos, and hepatosplenomegaly; similar cases were reported by Henry Christian and Arthur Schuller.[3,4] The disease was named Hand-Schuller-Christian disease. Based on similar histopathological patterns, Hand-Schuller-Christian disease, Letterer-Siwe disease, and eosinophilic granuloma were unified as histiocytosis X in 1953 and renamed LCH in 1985.[5] Histopathologically, LCH is generally defined by CD1a+/Langerin+ Langerhans-like cells. The specific origin of LCH cells has yet to be identified. The incidence of LCH is reported to be 3-5/million; the majority of patients are children younger than 3 years and the incidence in adults is approximately 1-2/million.[6] Incidence appears to be higher in Caucasians in northern European countries, and lower in Asia and Africa.[7]

The clinical manifestation of LCH varies from a single-organ disease that could spontaneously go into remission, to a systemic

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Case report

Veno-venous extracorporeal membrane oxygenation bridged living-donor lung transplantation for rapid progressive respiratory failure with pleuroparenchymal fibroelastosis after allogeneic hematopoietic stem cell transplantation

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ABSTRACT

Cases of extracorporeal membrane oxygenation (ECMO) bridged lung transplantation (LTx) are rare in Japan because an allocation system to prioritize patients based on urgency remains to be established. For critically ill patients who cannot wait for a brain-dead donor LTx, ECMO bridge to living-donor LTx may be the only practical option. A 21-year-old woman with pleuroparenchymal fibroelastosis after hematopoietic stem cell transplantation was admitted to our hospital with rapidly progressive respiratory failure. She was waitlisted for 6 months before admission, but veno-venous ECMO was initiated. She was

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Case report

Tocilizumab-effective multicentric Castleman's disease with infiltration of eosinophil and IgG_4-positive plasma cells: A case report

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ABSTRACT

A 67-year-old woman with fever and cough was diagnosed with eosinophilic pneumonia because of eosinophilia and increased eosinophil levels in the bronchoalveolar lavage fluid and transbronchial biopsy lung specimens. However, prednisolone therapy at a previous hospital was ineffective. Histological findings from thoracoscopic lung and lymph node biopsies were consistent with multicentric Castleman's disease (MCD). Since specimens also showed prominent eosinophil and IgG_4-positive plasma cell infiltration, it was difficult to distinguish IgG_4-related disease (IgG_4-RD) from MCD. Administration of prednisolone plus tocilizumab improved the symptoms and lung lesions, and prednisolone administration was successfully reduced and then terminated. The present case highlights the difficulty in diagnosing MCD and IgG_4-RD, and suggests that combined administration of tocilizumab and prednisolone might be effective in such a case.

1. Introduction

Multicentric Castleman's disease (MCD) is a benign lymphoproliferative disorder presenting with multiple enlarged lymph nodes associated with plasma cell invasion, and is characterized by polyclonal hypergammaglobulinemia due to IL-6 overproduction [1,2]. IgG_4-related disease (IgG_4-RD) is a novel disease entity characterized by high serum IgG_4 levels and tissue infiltration of IgG_4-producing plasma cells, and occasionally by eosinophilia and tissue eosinophil infiltration [3]. Since these diseases exhibit similar pathological findings, it can be difficult to differentiate MCD from IgG_4-RD [4–6]. Here, we report a tocilizumab-effective case that was initially diagnosed with eosinophilic pneumonia (EP), but was later diagnosed with MCD, with difficulty in excluding IgG_4-RD.

2. Case report

A 67-year-old woman with fever and cough was referred to a general hospital. A chest computed tomography (CT) scan revealed mediastinal lymphadenopathy and ground glass opacities in both lung fields. Initial blood examinations revealed a white blood cell (WBC) count of 11700/μL and an eosinophil count of 2925/μL. Cellular analysis of the bronchoalveolar lavage fluid (BALF) revealed 12.5% eosinophils. Histological findings from transbronchial lung biopsy (TBLB) specimens showed eosinophilic infiltration (5 cells/high-powered field [HPF]) (Fig. 1a). The patient was initially diagnosed with eosinophilic pneumonia, and oral prednisolone (PSL) was started at 30 mg/day. Thereafter, the ground glass opacities partially disappeared, and PSL was reduced to 10 mg/day. However, infiltrative opacities started appearing in the right middle lobe and the left lingula segment in chest CT. The patient was referred to our department for further examination.

Her medical history included steroid diabetes mellitus, surgery for extra-uterine pregnancy at the age of 30 years, and retinal detachment surgery at the age of 53. She had smoked four cigarettes a day for 20 years. She was receiving PSL 10 mg/day (prescribed for EP by the previous doctor), famotidine 20 mg/day, carbocysteine 1500 mg/day, and insulin lispro (8 U/day) for steroid diabetes mellitus. Her body temperature was 35.9 °C and her oxygen saturation was 98% on room air. Fine crackles were heard in the bilateral lower lungs, without wheezing. Superficial lymph nodes and submandibular glands were not palpable. She had no obvious symptoms of dry eyes, dry mouth, eruption, or numbness in the extremities.

Abbreviations: BALF, bronchoalveolar lavage fluid; CRP, C-reactive protein; CT, computed tomography; EGPA, eosinophilic granulomatosis with polyangiitis; EP, eosinophilic pneumonia; HPF, high-powered field; IgG_4-RD, IgG_4-related disease; MCD, multicentric Castleman's disease; PSL, prednisolone; TBLB, transbronchial lung biopsy; UCD, unicentric Castleman's disease; WBC, white blood cell

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