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HLA class I allele-lacking leukocytes predict rare clonal evolution to MDS/AML in patients with acquired aplastic anemia

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Abstract:

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A frequent nonsense mutation in exon 1 across certain HLA-A and -B alleles in leukocytes of patients with acquired aplastic anemia

by Hiroki Mizumaki, Kazuyoshi Hosomichi, Kohei Hosokawa, Takeshi Yoroidaka, Tatsuya Imi, Yoshitaka Zaimoku, Takamasa Katagiri, Mai Anh Thi Nguyen, Dung Cao Tran, Mahmoud Ibrahim Yousef Elbadry, Kazuhisa Chonabayashi, Yoshinori Yoshida, Hiroyuki Takamatsu, Tatsuhiko Ozawa, Fumihiro Azuma, Hiroyuki Kishi, Yoichi Fujii, Seishi Ogawa, Atsushi Tajima, and Shinji Nakao

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LETTER

Stem cell biology

High-dose romiplostim accelerates hematologic recovery in patients with aplastic anemia refractory to eltrombopag

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To the Editor:

Patients with acquired aplastic anemia (AA) refractory to eltrombopag (EPAG) have been reported to respond poorly to another thrombopoietin receptor agonist (TPO-RA), romiplostim (ROMI), in doses of up to 10 μg/kg per week. We analyzed the effectiveness of 20 μg/kg ROMI in 21 patients with EPAG-refractory AA. Sixteen of 21 (76%) achieved a hematologic response in at least one lineage at 3 months. Five of ten (50%) patients became independent of platelet ($n = 2$) or red cell ($n = 3$) transfusions. All 21 patients tolerated ROMI and showed no severe treatment-related adverse events that necessitated ROMI discontinuation. This retrospective study is the first to demonstrate high-dose ROMI was highly effective in AA patients refractory to EPAG.

AA is caused by immune-mediated destruction of hematopoietic stem and progenitor cells (HSPCs), resulting in bone marrow hypoplasia and pancytopenia in the peripheral blood [1]. Immunosuppressive therapy (IST) with horse anti-thymoglobulin (ATG) and cyclosporine (CsA) is the standard of care for patients with severe AA (SAA) [2]. However, ~30% of patients fail to respond, and therapeutic options for refractory cases are limited.

EPAG, a TPO-RA, has been shown to induce hematologic recovery in about 50% of patients with AA refractory to IST [3–6]. Moreover, EPAG was reported to increase response rates when added to ATG/CsA in treatment-naive SAA patients compared with a historical cohort [7]. Although EPAG has changed the paradigm of AA treatment, novel therapies are still needed to rescue EPAG-refractory patients. A recent clinical trial showed that another TPO-RA, ROMI, was effective in ~80% of EPAG-naïve patients with refractory SAA or non-severe AA (NSAA) when administered in doses of up to 20 μg/kg per week [8]. On the other hand, a retrospective study from France showed that only 1 of 8 SAA patients who received ROMI up to 10 μg/kg after failing EPAG improved, suggesting a limited role for ROMI in the treatment of EPAG-refractory SAA [9]. The effectiveness of 20 μg/kg ROMI (the maximum dose approved for use in AA patients in Japan) in EPAG-refractory cases has not been closely examined.

To determine the effectiveness of ROMI in patients who showed no or only a marginal response to EPAG, we retrospectively analyzed the response to ROMI in 21 AA patients and investigated pretreatment variables associated with a response to ROMI. Table 1 and Supplemental Table 1 show patient characteristics. Pretreatment blood cell counts of the 21 patients were as follows: neutrophils 0.16–2.33 × 10^9/L, (median: 0.83 × 10^9/L), Hb 5.2–12.7 g/dL (median: 7.5 g/dL), absolute reticulocytes 1–72 × 10^9/L (median: 37 × 10^9/L), and platelets 2–50 × 10^9/L (median: 16 × 10^9/L). The male to female ratio was 5:16 and median age at ROMI initiation was 55 years (range: 24–73 years). Six of the 21 patients (29%) had previously received at least one course of ATG, before starting ROMI. All 21 had previously been treated with EPAG at a dose of 75–100 mg/day (median: 100 mg/day, the maximum dose approved in Japan) for 2–56 (median: 20) months. EPAG (100 mg/day) was changed to ROMI after 2 months in one patient (Case 10) due to progression of cytopenia. Ten patients were transfusion-dependent

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後天性赤芽球癆の病態と治療研究の進歩

石田文宏

後天性赤芽球癆は正球性貧血，網赤血球減少と骨髄赤芽球系細胞の著減を認める造血障害性疾患である。背景疾患は多岐にわたり，急性の赤芽球癆との判別も含め鑑別は丁寧に行う必要がある。新規の背景要因として免疫チェックポイント阻害薬使用例が報告された。免疫病態として細胞性免疫異常やABO major不適合同種造血幹細胞移植後などでの液性免疫の関与が以前より指摘されている。特発性赤芽球癆を含め4割の症例で細胞傷害性T細胞に*STAT3*遺伝子変異を伴っていることが最近判明した。初期治療は赤血球輸血と免疫抑制療法が主体で，本邦ではシクロスポリン使用例が多い。8割で奏効するが再燃・不応の場合もある。複数薬剤に不応の場合は同種造血幹細胞移植も考慮されるが確立した方法はない。長期予後など諸課題解決のため，2016年から特発性造血障害に関する調査研究班による後天性慢性赤芽球癆の前向き登録研究が開始された。（臨床血液61（9）：1098～1104，2020）

Key words : Erythropoiesis, Cytotoxic T cell, Immunosuppressive therapy, STAT3

赤芽球癆（pure red cell aplasia, PRCA）は正球性貧血，網赤血球減少と骨髄赤芽球系細胞著減を認めるが，白血球，血小板の減少は伴わないことを特徴とする造血障害性疾患であり，種々の基礎疾患によって生ずるためひとつの症候群として考える必要がある[1,2]。難治性疾患克服事業：特発性造血障害に関する調査研究班より赤芽球癆診療の参照ガイド令和1年改訂版（第6版）が先頃公表されている[3]。本稿では後天性赤芽球癆について同ガイドに準拠し，最近の知見を含めて概説する。

疫学と分類（Table 1）

後天性赤芽球癆は比較的稀で年間新規発症数は人口100万人あたり0.3人程度と考えられる。日本血液学会の疾患登録では年間100例程度の新規症例が登録されている。

後天性赤芽球癆は経過により急性型と慢性型に分類され，急性型には主たる原因として薬剤性と感染性がある。慢性型は特発性と続発性があり，続発性の基礎疾患には，固形腫瘍として胸腺腫，T細胞リンパ腫として大顆粒リンパ球性（LGL）白血病，また，慢性リンパ性白血病，マクログロブリン血症，monoclonal gammopathy of undetermined significanceといったB細胞系腫瘍[4]や種々の自己免疫疾患が報告されている。頻度より，胸腺腫関連，LGL白血病関連，自己免疫性疾患関連，B細胞性腫瘍関連などに大別され，これらの続発性の原因が明らかでない場合，特発性赤芽球癆となる。後天性赤芽球癆は基礎疾患の有無およびその内容により対応や治療法が異なるので鑑別を丁寧に行う必要がある。

造血障害を来す疾患のうち，骨髄異形成症候群（MDS）で赤芽球造血が著しく低下している例では赤芽球癆と判別困難な骨髄像を呈することもある。また，赤芽球癆からほかの血球減少を合併し再生不良性貧血へと変化する例もあり，赤芽球癆とこれら造血障害を来す疾患は密接に関連・重複しているともいえ，明確に区別せず一連の造血不全としてとらえる考えもある。

病因・病態

急性感染性の赤芽球癆で，例えば，ヒトパルボウイルス（HPV）B19感染による場合には赤血球前駆細胞にウイルスが血液型P抗原を介して直接感染し，赤血球造血を障害することが判明している。ほかの微生物感染関連赤芽球癆の報告もあるが，病態の詳細は明らかになっていない。

特発性を含め慢性型の多くの場合には，赤血球造血が免疫異常により障害される可能性が示されてきた。

液性免疫の関与として，赤芽球系前駆細胞に対する抗体が赤芽球癆の原因として明らかであるのが，ABO major不適合ドナーから同種造血幹細胞移植を受けた症例に発

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Clonal hematopoiesis in adult pure red cell aplasia

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Idiopathic pure red cell aplasia (PRCA) and secondary PRCA associated with thymoma and large granular lymphocyte leukemia are generally considered to be immune-mediated. The PRCA2004/2006 study showed that poor responses to immunosuppression and anemia relapse were associated with death. PRCA may represent the prodrome to MDS. Thus, clonal hematopoiesis may be responsible for treatment failure. We investigated gene mutations in myeloid neoplasm-associated genes in acquired PRCA. We identified 21 mutations affecting amino acid sequences in 11 of the 38 adult PRCA patients (28.9%) using stringent filtering of the error-prone sequences and SNPs. Four PRCA patients showed 7 driver mutations in *TET2*, *DNMT3A* and *KDM6A*, and 2 PRCA patients carried multiple mutations in *TET2*. Five PRCA patients had mutations with high VAFs exceeding 0.3. These results suggest that clonal hematopoiesis by stem/progenitor cells might be related to the pathophysiology of chronic PRCA in certain adult patients.

Idiopathic pure red cell aplasia (PRCA) and secondary PRCA not responding to treatments for the underlying diseases in adults are generally considered to be immune-mediated and are treated by immunosuppressive therapy[1,2]. We previously conducted the PRCA2004/2006 study and reported that poor responses to induction therapy and anemia relapse were associated with death[3–6]. Principal causes of death were infections and organ failure. Different outcomes in adult PRCA patients depending on their responses to immunosuppression suggest the heterogeneity of chronic PRCA in adults. Based on previous findings, idiopathic PRCA may be the prodrome to myelodysplastic syndromes[7,8]. In some cases, erythroid hypoplasia/aplasia has been observed in patients with myelodysplastic syndrome (MDS)[9–11].

Theoretically, there are two potential mechanisms of unresponsiveness to immunosuppression: clonal changes in autoaggressive lymphocytes reacting against erythroid progenitors and clonal hematopoiesis by stem/progenitor cells that have undergone somatic mutations during the disease progression of PRCA. Regarding the former, mutations in the signal transducer and activator of the transcription 3 gene (*STAT3*) were detected in 40% of patients with large granular lymphocyte (LGL) leukemia[12] and have been found in PRCA[13], aplastic anemia, and MDS patients[14]. Kawakami et al. recently reported that *STAT3* mutations were detected in 43% of PRCA

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Successful treatment of a PNH patient non-responsive to eculizumab with the novel complement C5 inhibitor coversin (nomacopan)

Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare acquired life-threatening disease characterised by complement-induced haemolysis and a high incidence of thrombosis. The monoclonal antibody eculizumab binds to complement C5 and prevents its activation and cleavage into C5a and C5b. Treatment of PNH patients with eculizumab decreases haemolysis, transfusion requirements and the risk of thrombosis (Hillmen *et al.*, 2004, 2006, 2007; Brodsky *et al.*, 2008; Kelly *et al.*, 2011). Failure to respond to eculizumab has been reported in a subgroup of Asian PNH patients (Nishimura *et al.*, 2014). These patients had a genetic variant of C5, a missense mutation leading to c.2654G>A (p.Arg885His), which occurs in approximately 3·5% of Japanese and 1% of Chinese Han populations. This genetic variant interferes with the binding of eculizumab to C5 (Nishimura *et al.*, 2014). For PNH patients with this genetic variant there is no effective treatment.

A new small (17 kDa) protein complement inhibitor named coversin is now in phase 2 clinical development. Like eculizumab, coversin prevents cleavage and activation of C5,

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Beneficial effects of eculizumab regardless of prior transfusions or bone marrow disease: Results of the International Paroxysmal Nocturnal Hemoglobinuria Registry

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Funding information
Alexion Pharmaceuticals, Inc.

Abstract

Objectives: To evaluate the effects of eculizumab on transfusions and thrombotic events (TEs) in patients with and without prior history of transfusion in the International Paroxysmal Nocturnal Hemoglobinuria (PNH) Registry.

Methods: Registry patients enrolled on or before January 1, 2018, initiated on eculizumab no more than 12 months prior to enrollment, having known transfusion status for the 12 months before eculizumab initiation, and ≥12 months of Registry follow-up after eculizumab initiation, were included.

Results: Eculizumab treatment was associated with a 50% reduction in transfusions in patients with a transfusion history (10.6 units/patient-year before eculizumab vs 5.4 after; $P < .0001$), with greater reduction observed in those with no history of bone marrow disease vs those with bone marrow disease. Mean lactate dehydrogenase levels decreased from a mean of 6.7 to 1.4 times the upper limit of normal (ULN) in patients with transfusion history and from 5.1 to 1.2 times ULN in those with no transfusion history. TE and major adverse vascular event rates also decreased by 70% in patients with and without history of transfusion.

Conclusions: The benefit of eculizumab therapy does not appear to be limited to any group defined by transfusion history or bone marrow disease history.

KEYWORDS

aplastic anemia, blood transfusion, bone marrow diseases, eculizumab, paroxysmal hemoglobinuria, registries, thrombosis

1 | INTRODUCTION

The clinical manifestations of paroxysmal nocturnal hemoglobinuria (PNH) arise from the uncontrolled activation of the terminal complement pathway and associated intravascular hemolysis, which is the major contributor to mortality and morbidity.[1-3] PNH is characterized by hemolytic anemia and can be further complicated by concomitant bone marrow diseases such as aplastic anemia and myelodysplastic syndrome.[4,5]

Historically, red blood cell (RBC) transfusion dependence was considered a key marker of disease severity in patients with PNH, and RBC transfusions were frequently used as first-line treatment to manage anemia.[6,7] In fact, transfusion dependence was an inclusion criterion for patients enrolled in the pivotal studies of eculizumab

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ORIGINAL ARTICLE

Baseline clinical characteristics and disease burden in patients with paroxysmal nocturnal hemoglobinuria (PNH): updated analysis from the International PNH Registry

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Abstract

The International Paroxysmal Nocturnal Hemoglobinuria (PNH) Registry (NCT01374360) was initiated to optimize patient management by collecting data regarding disease burden, progression, and clinical outcomes. Herein, we report updated baseline demographics, clinical characteristics, disease burden data, and observed trends regarding clone size in the largest cohort of Registry patients. Patients with available data as of July 2017 were stratified by glycosylphosphatidylinositol (GPI)-deficient granulocyte clone size (< 10%, ≥ 10%–< 50%, and ≥ 50%). All patients were untreated with eculizumab at baseline, defined as date of eculizumab initiation or date of Registry enrollment (if never treated with eculizumab). Outcomes assessed in the current analysis included proportions of patients with high disease activity (HDA), history of major adverse vascular events (MAVEs; including thrombotic events [TEs]), bone marrow failure (BMF), red blood cell (RBC) transfusions, and PNH-related symptoms. A total of 4439 patients were included, of whom 2701 (60.8%) had available GPI-deficient granulocyte clone size data. Among these, median clone size was 31.8% (1002 had < 10%; 526 had ≥ 10%–< 50%; 1173 had ≥ 50%). There were high proportions of patients with HDA (51.6%), history of MAVEs (18.8%), BMF (62.6%), RBC transfusion (61.3%), and impaired renal function (42.8%). All measures except RBC transfusion history significantly correlated with GPI-deficient granulocyte clone size. A large proportion of patients with GPI-deficient granulocyte clone size < 10% had hemolysis (9.7%), MAVEs (10.2%), HDA (9.1%), and/or PNH-related symptoms. Although larger GPI-deficient granulocyte clone sizes were associated with higher disease burden, a substantial proportion of patients with smaller clone sizes had history of MAVEs/TEs.

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LETTERS

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Implications of *TP53* allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes

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Tumor protein p53 (*TP53*) is the most frequently mutated gene in cancer[1,2]. In patients with myelodysplastic syndromes (MDS), *TP53* mutations are associated with high-risk disease[3,4], rapid transformation to acute myeloid leukemia (AML)[5], resistance to conventional therapies[6–8] and dismal outcomes[9]. Consistent with the tumor-suppressive role of *TP53*, patients harbor both mono- and biallelic mutations[10]. However, the biological and clinical implications of *TP53* allelic state have not been fully investigated in MDS or any other cancer type. We analyzed 3,324 patients with MDS for *TP53* mutations and allelic imbalances and delineated two subsets of patients with distinct phenotypes and outcomes. One-third of *TP53*-mutated patients had monoallelic mutations whereas two-thirds had multiple hits (multi-hit) consistent with biallelic targeting. Established associations with complex karyotype, few co-occurring mutations, high-risk presentation and poor outcomes were specific to multi-hit patients only. *TP53* multi-hit state predicted risk of death and leukemic transformation independently of the Revised International Prognostic Scoring System (IPSS-R)[11]. Surprisingly, monoallelic patients did not differ from *TP53* wild-type patients in outcomes and response to therapy. This study shows that consideration of *TP53* allelic state is critical for diagnostic and prognostic precision in MDS as well as in future correlative studies of treatment response.

In collaboration with the International Working Group for Prognosis in MDS (Supplementary Table 1), we assembled a cohort of 3,324 peridiagnostic and treatment-naive patients with MDS or closely related myeloid neoplasms (Extended Data Fig. 1 and Supplementary Fig. 1). Genetic profiling included conventional G-banding analyses (CBA) and tumor-only, capture-based, next-generation sequencing (NGS) of a panel of genes recurrently mutated in MDS, as well as genome-wide copy number probes. Allele-specific copy number profiles were generated from NGS data using the CNACS algorithm[7] (see Methods and Code availability). An additional 1,120 samples derived from the Japanese MDS consortium (Extended Data Fig. 2) were used as a validation cohort.

To study the effect of *TP53* allelic state on genome stability, clinical presentation, outcome and response to therapy, we performed a detailed characterization of alterations at the *TP53* locus. First, we assessed genome-wide allelic imbalances in the cohort of 3,324 patients, to include arm-level or focal (~3 Mb) ploidy alterations and regions of copy-neutral loss of heterozygosity (cnLOH) (Extended Data Fig. 3, Supplementary Figs. 2–4 and Methods).

A full list of affiliations appears at the end of the paper.

blood

Regular Article

HEMATOPOIESIS AND STEM CELLS

SLFN11 promotes stalled fork degradation that underlies the phenotype in Fanconi anemia cells

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KEY POINTS

- **A DNA damage sensitizing gene *SLFN11* promotes stalled fork degradation caused by DNA2 and MRE11 nucleases by inhibiting RAD51 accumulation.**
- **Suppression of *SLFN11* in Fanconi anemia cells attenuates FA phenotypes such as chromosomal breakages or cell cycle arrest on DNA damage.**

Fanconi anemia (FA) is a hereditary disorder caused by mutations in any 1 of 22 FA genes. The disease is characterized by hypersensitivity to interstrand crosslink (ICL) inducers such as mitomycin C (MMC). In addition to promoting ICL repair, FA proteins such as RAD51, BRCA2, or FANCD2 protect stalled replication forks from nucleolytic degradation during replication stress, which may have a profound impact on FA pathophysiology. Recent studies showed that expression of the putative DNA/RNA helicase SLFN11 in cancer cells correlates with cell death on chemotherapeutic treatment. However, the underlying mechanisms of SLFN11-mediated DNA damage sensitivity remain unclear. Because *SLFN11* expression is high in hematopoietic stem cells, we hypothesized that *SLFN11* depletion might ameliorate the phenotypes of FA cells. Here we report that *SLFN11* knockdown in the FA patient-derived *FANCD2*-deficient PD20 cell line improved cell survival on treatment with ICL inducers. *FANCD2*$^{-/-}$*SLFN11*$^{-/-}$ HAP1 cells also displayed phenotypic rescue, including reduced levels of MMC-induced chromosome breakage compared with *FANCD2*$^{-/-}$ cells. Importantly, we found that *SLFN11* promotes extensive fork degradation in *FANCD2*$^{-/-}$ cells. The degradation process is mediated by the nucleases MRE11 or DNA2 and depends on the SLFN11 ATPase activity. This observation was accompanied by an increased RAD51 binding at stalled forks, consistent with the role of RAD51 antagonizing nuclease recruitment and subsequent fork degradation. Suppression of *SLFN11* protects nascent DNA tracts even in wild-type cells. We conclude that *SLFN11* destabilizes stalled replication forks, and this function may contribute to the attrition of hematopoietic stem cells in FA. (*Blood*. 2021;137(3):336-348)

Introduction

The *Schlafen* (*SLFN*) family was first described as a set of homologous genes that are involved in T-cell development and inhibit cell growth.[1] These genes are found only in mammals and a small number of nonmammalian species.[2] A member of the SLFN family, SLFN11, is a putative DNA/RNA helicase ubiquitously expressed in the human body.[3] Notably, its expression is often lost in primary cancers and in commonly used cancer cell lines by epigenetic silencing.[4,5] Recent studies indicated that SLFN11 expression in cancer correlate with a favorable response to widely used anticancer drugs, such as irinotecan, cisplatin, etoposide, and poly(ADP-ribose) polymerase inhibitors, with a better prognosis for the patients.[5-7] *SLFN11* expression has the strongest association among the DNA repair proteins with the sensitivities to DNA damaging cancer chemotherapy drugs but not to non-DNA damaging agents.[8] It has also been reported that SLFN11 associates with replication protein A (RPA) and negatively regulates RPA loading to chromatin, thereby disfavoring homologous recombination (HR) repair.[9] Another study found that SLFN11 accumulates at stalled replication forks and blocks replication.[10] SLFN11 may also affect protein translation by cleavage of a specific group of tRNAs, resulting in the abrogation of ATR kinase expression during the DNA damage response.[11,12] These studies define SLFN11 as a guardian of the genome that controls cell fate decisions in response to DNA damage and replication stress. However, how *SLFN11* exerts this function remains unclear.

Fanconi anemia (FA) is a rare hereditary disorder that is caused by mostly recessive mutations in any 1 of 22 FA genes identified thus far (*FANCA-W*), leading to hematopoietic stem cell failure and cancer predisposition.[13,14] FA proteins act in the common FA pathway to repair interstrand crosslink (ICL) damage, and therefore FA cells are hypersensitive to ICL-inducing agents such

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RESEARCH ARTICLE

Combined Cohesin–RUNX1 Deficiency Synergistically Perturbs Chromatin Looping and Causes Myelodysplastic Syndromes

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Human Mutation HGVS HUMAN GENOME VARIATION SOCIETY WILEY

BRIEF REPORT

A founder variant in the South Asian population leads to a high prevalence of *FANCL* Fanconi anemia cases in India

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Abstract

Fanconi anemia (FA) is a rare genetic disorder characterized by bone marrow failure, predisposition to cancer, and congenital abnormalities. FA is caused by pathogenic variants in any of 22 genes involved in the DNA repair pathway responsible for removing interstrand crosslinks. FANCL, an E3 ubiquitin ligase, is an integral component of the pathway, but patients affected by disease-causing *FANCL* variants are rare, with only nine cases reported worldwide. We report here a *FANCL* founder variant, anticipated to be synonymous, c.1092G>A;p.K364=, but demonstrated to induce aberrant splicing, c.1021_1092del;p.W341_K364del, that accounts for the onset of FA in 13 cases from South Asia, 12 from India and one from Pakistan. We comprehensively illustrate the pathogenic nature of the variant, provide evidence for a founder effect, and propose including this variant in genetic screening of suspected

*Frank X. Donovan, Avani Solanki, and Minako Mori contributed equally to this work.

SCIENTIFIC REPORTS
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OPEN

Assessment of dysplasia in bone marrow smear with convolutional neural network

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In this study, we developed the world's first artificial intelligence (AI) system that assesses the dysplasia of blood cells on bone marrow smears and presents the result of AI prediction for one of the most representative dysplasia—decreased granules (DG). We photographed field images from the bone marrow smears from patients with myelodysplastic syndrome (MDS) or non-MDS diseases and cropped each cell using an originally developed cell detector. Two morphologists labelled each cell. The degree of dysplasia was evaluated on a four-point scale: 0–3 (e.g., neutrophil with severely decreased granules were labelled DG3). We then constructed the classifier from the dataset of labelled images. The detector and classifier were based on a deep neural network pre-trained with natural images. We obtained 1797 labelled images, and the morphologists determined 134 DGs (DG1: 46, DG2: 77, DG3: 11). Subsequently, we performed a five-fold cross-validation to evaluate the performance of the classifier. For DG1–3 labelled by morphologists, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were 91.0%, 97.7%, 76.3%, 99.3%, and 97.2%, respectively. When DG1 was excluded in the process, the sensitivity, specificity, PPV, NPV, and accuracy were 85.2%, 98.9%, 80.6%, and 99.2% and 98.2%, respectively.

Many attempts have been made in the past decade to automatically determine cell types in blood smears. Initially, researchers developed algorithms to detect leukocytes, red blood cells, or nuclear segmentation[1–10]. Subsequently, they started addressing the detection of abnormal leukocytes including various types of leukemic cells[11–17]. However, these works mainly focused on peripheral blood smears, and few studies have covered bone marrow due to their greater complexity[18–20]. As there are many types of progenitor cells with various stages of continuous maturation in bone marrow specimens, a microscopic field contains a larger amount of information compared to peripheral blood. Moreover, the examination of bone marrow smears requires morphological evaluation in clinical settings, whereas the examination of peripheral blood mainly focuses on cell counting. These hurdles have prevented the development of automated examination of bone marrow smears and delayed the application of machine learning technology in the diagnosis of bone marrow disorders.

Myelodysplastic syndrome (MDS) is a haematological disease that develops mainly in the elderly and is characterised by an abnormal morphology (dysplasia) of blood cells in bone marrow. Haematopoietic progenitor cells, which acquire certain somatic gene mutations, clonally expand in bone marrow, leading to cytopenia characterised by ineffective haematopoiesis with myelodysplasia. Progressive cytopenia in multiple blood lineages and transformation to acute myeloid leukaemia are causes of death in patients with MDS. The morphological examination of bone marrow smears using light microscopy plays a critical role in the diagnosis of MDS. Since the first report of this disease, various types of dysplasia in cell lineages have been identified, such as granulocyte, erythrocyte, and megakaryocyte. The presence of bone marrow dysplasia, which is a requisite condition for the

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bjh research paper

Efficacy and safety of romiplostim in refractory aplastic anaemia: a Phase II/III, multicentre, open-label study

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Abstract

A previous dose-finding study has suggested that romiplostim is effective in patients with refractory aplastic anaemia (AA) and 10 μg/kg once weekly was recommended as a starting dose. In this Phase II/III, multicentre, open-label study, romiplostim was administered subcutaneously at a fixed dose of 10 μg/kg once weekly for 4 weeks (weeks 1–4) followed by weekly doses (5, 10, 15 and 20 μg/kg) titrated by platelet response for up to 52 weeks (weeks 5–52). A total of 31 patients with AA who were refractory to immunosuppressive therapy (IST) and thrombocytopenia (platelet count of $\leq 30 \times 10^9$/l) were enrolled. The primary efficacy endpoint of the proportion of patients achieving any haematological (platelet, neutrophil and erythrocyte) response at week 27 was 84% [95% confidence interval (CI) 66–95%]. Trilineage response was 39% (95% CI 22–58%) at week 53. The most common treatment-related adverse events (AEs) were headache and muscle spasms (each 13%). All AEs were mild or moderate except for three patients with Grade 3 hepatic AEs; no AEs necessitated romiplostim discontinuation. Two patients developed cytogenetic abnormalities, of whom one returned to normal karyotype at last follow-up. High-dose romiplostim is effective and well tolerated in the treatment of patients with AA refractory to IST.

Keywords: aplastic anaemia, bone marrow failure, haematopoiesis, thrombopoietin.

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[ORIGINAL ARTICLE]

Eltrombopag in Combination with Rabbit Anti-thymocyte Globulin/Cyclosporine A in Immunosuppressive Therapy-naïve Patients with Aplastic Anemia in Japan

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Abstract:

Objective In Japan, immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG), and cyclosporine A (CsA) is the standard of care in patients with aplastic anemia (AA) who are not indicated for stem-cell transplantation, although some patients may experience relapse. This study assessed the efficacy and safety of eltrombopag in combination with rabbit-ATG/CsA in IST-naïve patients with non-severe or severe AA in Japan.

Methods In this non-randomized, open-label, single-arm, phase II study, rabbit-ATG/CsA and eltrombopag were initiated on Days 1 and 15 (±3 days), respectively, and continued for ≥26 weeks; rabbit-ATG was given for 5 days (Days 1 to 5). The primary endpoint was the overall response rate (ORR) at Week 26.

Patients Patients with AA who were IST-naïve and ≤70 years old or between 71 and 75 years old based on the recommendation of the investigator were enrolled in Japan.

Results Of the 11 enrolled patients, 10 started treatment with eltrombopag. The ORRs at Weeks 26 and 52 were 70.0% and 60.0%, respectively. The ORR at Week 26 was 100% (all 3 patients) in patients with non-severe AA and 57.1% (4/7) in patients with severe AA. Among transfusion-dependent patients, 66.7% (4/6) and 62.5% (5/8) became red blood cell- and platelet-transfusion independent, respectively. The most common adverse events were nausea and headache. No deaths or hematologic malignancies were reported. A cytogenetic abnormality was reported in one patient.

Conclusion This study confirmed the clinical benefit of eltrombopag plus rabbit-ATG/CsA in IST-naïve patients with non-severe or severe AA in Japan.

Key words: eltrombopag, aplastic anemia, rabbit-ATG/CsA, Japan

(Intern Med Advance Publication)
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Introduction

Aplastic anemia (AA) is a bone marrow failure disorder characterized by pancytopenia and hypocellular marrow (1, 2). It is most likely caused by an immune-mediated mechanism through T cells, resulting in a marked reduction in hematopoietic stem cells (3-5). A survey data reported that the incidence of AA was 8.2 per million person-years from 2004 to 2012 (6). In Europe and North America, the

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Prognostic impact of peripheral blood *Wilms' tumour 1* mRNA expression levels in response to azacytidine in MDS: A single-centre analysis

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ARTICLE INFO

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Myelodysplastic Syndromes
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ABSTRACT

To determine the impact of peripheral blood (PB) *Wilms' tumour 1* (*WT-1*) mRNA levels in patients with primary myelodysplastic syndromes (MDS), we analysed the relationships between several clinical variables at the time of diagnosis and the haematological response of patients treated with azacytidine. We observed overall responses in 20 (63%) patients; there were no significant differences in clinical variables, including bone marrow blast counts, IPSS scores and IPSS-R risk scores, between responders and non-responders. The responders' PB *WT-1* mRNA levels were significantly lower than those of non-responders ($P = 0.03$). PB *WT-1* mRNA expression could be a marker for predicting the response to azacytidine in patients with *de novo* MDS.

1. Introduction

Myelodysplastic syndromes (MDS) are heterogeneous disorders characterised by cytopenia with dysplasia and a propensity to progress to acute myeloid leukaemia [1, 2]. Several prognostic scoring systems for MDS have been reported, including the International Prognostic Scoring System (IPSS), the World Health Organisation (WHO) Prognostic Scoring System (WPSS) and the revised IPSS (IPSS-R) [3–5]. Azacytidine, a hypomethylating agent, has been demonstrated to induce responses, delay leukaemic transformation and improve survival in higher-risk MDS. Because haematological response rates are not as high in patients treated with azacytidine, it is important to identify patients with MDS who respond to azacytidine. However, the predictors of haematologic response in patients with MDS taking azacytidine have yet to be fully determined.

The survival of patients with MDS has been associated with the expression of several genes, including *LEF1, CDH1, Wilms' tumour 1* gene (*WT-1*) and *MN1*, and the expression levels of *WT-1* were higher in patients with MDS with poor survival [6]. Although *WT-1* was initially identified as a tumour suppressor gene in patients with Wilms' tumour, recent results indicated that *WT-1* acts as an oncogene in various solid tumours and haematological malignancies [7]. *WT-1* levels in bone marrow (BM) could be useful for predicting the survival of patients with myeloid neoplasms treated with azacytidine [8]. *WT-1* expression levels in peripheral blood (PB) have proven useful for assessing the risk in patients with MDS [9]. PB sampling has multiple distinct benefits over BM sampling such as more frequent PB collection from the same patient than BM. *WT-1* levels in PB reflect the disease progression of patients with MDS treated with hypomethylating agents [10]. However, the relationship between *WT-1* levels in PB and the response to azacytidine treatment is unclear; moreover, the prognostic role of PB *WT-1* levels in primary MDS has not been fully established.

To obtain a more complete insight into the prognostic value of PB *WT-1* levels in primary MDS, we analysed the relationships between several clinical variables (including *WT-1* mRNA expression levels) at the time of diagnosis (baseline) and the haematological response (best response) of patients with MDS to azacytidine and elucidated the impact of this response on their overall survival.

2. Materials and methods

2.1. Patients

Patients with MDS (according to the French-American-British classification) who were referred to Saitama International Medical Centre (Saitama Medical University, Saitama, Japan) between July 2011 and

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ORIGINAL ARTICLE

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Efficacy of the novel tubulin polymerization inhibitor PTC-028 for myelodysplastic syndrome

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Abstract

Monomer tubulin polymerize into microtubules, which are highly dynamic and play a critical role in mitosis. Therefore, microtubule dynamics are an important target for anticancer drugs. The inhibition of tubulin polymerization or depolymerization was previously targeted and exhibited efficacy against solid tumors. The novel small molecule PTC596 directly binds tubulin, inhibits microtubule polymerization, down-regulates MCL-1, and induces p53-independent apoptosis in acute myeloid leukemia cells. We herein investigated the efficacy of PTC-028, a structural analog of PTC596, for myelodysplastic syndrome (MDS). PTC-028 suppressed growth and induced apoptosis in MDS cell lines. The efficacy of PTC028 in primary MDS samples was confirmed using cell proliferation assays. PTC-028 synergized with hypomethylating agents, such as decitabine and azacitidine, to inhibit growth and induce apoptosis in MDS cells. Mechanistically, a treatment with PTC-028 induced G2/M arrest followed by apoptotic cell death. We also assessed the efficacy of PTC-028 in a xenograft mouse model of MDS using the MDS cell line, MDS-L, and the AkaBLI bioluminescence imaging system, which is composed of AkaLumine-HCl and Akaluc. PTC-028 prolonged the survival of mice in xenograft models. The present results suggest a chemotherapeutic strategy for MDS through the disruption of microtubule dynamics in combination with DNA hypomethylating agents.

KEYWORDS

Tubulin polymerization inhibitor, chemotherapy, DNA hypomethylating agents, Myelodysplastic syndrome

Cheng Zhong and Kensuke Kayamori contributed equally to this work.

REGULAR ARTICLE

blood advances

DHODH inhibition synergizes with DNA-demethylating agents in the treatment of myelodysplastic syndromes

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Key Points

- DHODH inhibition synergizes with DNA-demethylating agents in the treatment of MDS.
- DHODH inhibition enhances the incorporation of decitabine into DNA in MDS cells.

Dihydroorotate dehydrogenase (DHODH) catalyzes a rate-limiting step in de novo pyrimidine nucleotide synthesis. DHODH inhibition has recently been recognized as a potential new approach for treating acute myeloid leukemia (AML) by inducing differentiation. We investigated the efficacy of PTC299, a novel DHODH inhibitor, for myelodysplastic syndrome (MDS). PTC299 inhibited the proliferation of MDS cell lines, and this was rescued by exogenous uridine, which bypasses de novo pyrimidine synthesis. In contrast to AML cells, PTC299 was inefficient at inhibiting growth and inducing the differentiation of MDS cells, but synergized with hypomethylating agents, such as decitabine, to inhibit the growth of MDS cells. This synergistic effect was confirmed in primary MDS samples. As a single agent, PTC299 prolonged the survival of mice in xenograft models using MDS cell lines, and was more potent in combination with decitabine. Mechanistically, a treatment with PTC299 induced intra-S-phase arrest followed by apoptotic cell death. Of interest, PTC299 enhanced the incorporation of decitabine, an analog of cytidine, into DNA by inhibiting pyrimidine production, thereby enhancing the cytotoxic effects of decitabine. RNA-seq data revealed the marked downregulation of MYC target gene sets with PTC299 exposure. Transfection of MDS cell lines with *MYC* largely attenuated the growth inhibitory effects of PTC299, suggesting *MYC* as one of the major targets of PTC299. Our results indicate that the DHODH inhibitor PTC299 suppresses the growth of MDS cells and acts in a synergistic manner with decitabine. This combination therapy may be a new therapeutic option for the treatment of MDS.

Introduction

Myelodysplastic syndrome (MDS) is a clonal bone marrow (BM) disorder characterized by ineffective and clonal hematopoiesis accompanied by morphological dysplasia and variable cytopenia. DNA methyltransferase inhibitors azacitidine and decitabine have been used as chemotherapeutic agents for high-risk MDS. They are chemical analogs of cytidine that have direct cytotoxicity and induce DNA hypomethylation by interfering with DNA methyltransferase. Overall survival has been prolonged in

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LETTER TO THE EDITOR

Faggot cells in acute myeloid leukemia with t(7;11)(p15;p15) and *NUP98-HOXA9* fusion

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Dear Editor,

Faggot cells are myeloid cells harboring multiple Auer rods that are randomly distributed in the cytoplasm, and these cells are considered diagnostic markers for acute promyelocytic leukemia (APL), although their biological significance remains unknown [1]. Here, we report a case of acute myeloid leukemia (AML) of the non-APL type which exhibited faggot cells.

A 62-year-old man presented with fever and general malaise. Blood test results were as follows: red blood cell count, 2.44 × 10^6/μL; hemoglobin, 8.1 g/dL; total leukocyte count, 4920/μL with 9% blasts; and platelet count, 149 × 10^3/μL. Bone marrow examination revealed extremely hypercellular marrow with increased immature myeloid cells and 46% blasts, 26% promyelocytes, and 9% myelocytes among all nucleated cells. Immature myeloid cells with Auer rods were also frequently observed, of which some contained multiple rods (Fig. 1). Flow cytometry analyses showed that the cells in a CD45-dull fraction were strongly positive for CD33 (96%), CD38 (97%), and CD117 (81%); weakly positive for CD13 (25%), CD34 (13%), myeloperoxidase (10%), and glycophorine A (23%); and negative for CD3, CD14, CD19, CD41, and HLA-DR.

According to the morphological features of the bone marrow cells, although there were no clinical signs of disseminated intravascular coagulation, the patient was first speculated to have APL, and thus, all-trans retinoic acid was prepared. However, the screening tests for fusion transcripts using quantitative real-time polymerase chain reactions revealed that the *PML-RARA* fusion transcripts were absent; however, *NUP98-HOXA9* fusion transcripts were amplified (3.7 × 10^4 copies/μg RNA). Consistent with these results, the chromosomal karyotype of the patient was 46, XY, t(7;11)(p15;p15) in all examined metaphases (Fig. 2), and *PML-RARA* fusion was absent in FISH analysis. The patient was treated with a conventional induction chemotherapy for AML, which successfully induced hematological remission.

Translocation t(7;11)(p15;p15) is an uncommon but recurrent chromosomal abnormality in myeloid neoplasms, with most cases reported in Asian countries [2]. In this translocation, the fusion of *NUP98* at chromosome 11 and *HOXA9* at chromosome 7 occurs [3, 4], and the product plays crucial roles in the pathogenesis of such neoplasms [5]. Morphologically, most cases are classified as AML M2 according to the French-American-British (FAB) classification, and the prognosis of these cases is

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赤血球系疾患
13（EL2-5C）

炎症性貧血の発症機構

川 端　浩

炎症は非特異的な生体防御反応であるが，これが長期にわたると貧血を生じる。その発症には免疫細胞から放出されるさまざまな液性因子が関与する。腫瘍壊死因子 α（TNFα）は造血幹細胞を白血球系に分化させる転写因子 PU.1 を増加させ，赤芽球系への分化を促す転写因子 GATA-1 の発現を低下させる。TNFα とインターロイキン 1β（IL-1β）は腎臓におけるエリスロポエチン産生を低下させ，インターフェロン γ は造血前駆細胞におけるエリスロポエチン受容体の発現を低下させる。IL-6 は肝臓におけるヘプシジン産生を増加させ，これが鉄のリサイクル・システムを阻害して造血系における鉄の利用障害を引き起こす。マクロファージの活性化により赤血球の寿命も短縮する。治療としては原疾患の改善が最も重要であるが，慢性腎臓病合併例では赤血球造血刺激薬が用いられ，関節リウマチ例などではヘプシジンの経路を抑制する抗 IL-6 受容体抗体も用いられる。（臨床血液 61（9）：1105～1111，2020）

Key words : Tumor necrosis factor-α, Interleukin-6, Erythropoietin, Hepcidin

はじめに

「炎症」は，組織の損傷や外敵の侵入に対処して宿主を守るために，生命が進化の過程で構築してきた非特異的な生体防御反応である。ヒトにおいては，炎症の中心的役割を担うのは好中球，リンパ球，樹状細胞，単球，マクロファージなどの免疫細胞で，これらが活性化し，さまざまなサイトカインを放出して炎症反応を遂行する。全身性に生じる炎症の基礎疾患としては，細菌，結核，真菌，ウイルスなどによる慢性感染症，関節リウマチや全身性エリテマトーデスなどの自己免疫疾患，潰瘍性大腸炎や Crohn 病などの炎症性腸疾患，Castleman 病，悪性腫瘍などがある。全身性の炎症が長期間持続すると，炎症性貧血（anemia of inflammation）をきたす。本稿では，炎症性貧血の機序を概説し，診断と治療についても触れたい。

1．貧血の発症機構

炎症では免疫細胞から放出されるインターロイキン 1β（interleukin-1β, IL-1β），IL-6，IL-10，腫瘍壊死因子 α（tumor necrosis factor α, TNFα），インターフェロン γ（interferon γ, IFNγ）などの炎症性サイトカインが複合して働いて炎症性貧血を引き起こす[1]。炎症性貧血の病態は複雑で複合的で未解明の部分も多いが，（1）血球系統を制御する転写因子のバランスの変化，（2）腎臓におけるエリスロポエチン（erythropoietin, EPO）産生の低下，（3）赤芽球系前駆細胞における EPO 受容体（EPO receptor, EPOR）の発現低下，（4）ヘモグロビン合成に必要な鉄の供給低下，（5）産生された赤血球の寿命の短縮といったさまざまな機序が考えられている（Fig. 1）。

（1）血球系統を制御する転写因子のバランスの変化

炎症が果たす重要な働きの一つは体内に侵入した病原体の排除であり，炎症はいわば外敵との戦争とも言える。生体は侵入した病原微生物に対抗するために，脾臓などに蓄えられていた好中球を一斉に動員する。骨髄では造血幹細胞から白血球への細胞分化が促進される。その一方で，酸素の運搬を担う赤血球の造血は後回しにされる。こういった造血系統の変化は，造血幹細胞における転写因子の発現制御によってもたらされる。すなわち，炎症性サイトカインの TNFα は，造血幹細胞を白血球系に分化させる転写因子 PU.1 を増加させる一方で[2]，赤血球系への分化を促す転写因子 GATA-1 の発現を低下させる[3]。GATA-1 の標的遺伝子には EPOR や，グロビン遺伝子群，ヘム合成に必須なアミノレブリン酸合成酵素（5-aminolevulinic acid synthase, ALAS）も含まれるため，GATA-1 の発現低下は赤血球造血を抑制し，貧血を引き起こす[4]。

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ORIGINAL ARTICLE

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No clear survival benefit of azacitidine for lower-risk myelodysplastic syndromes: A retrospective study of Nagasaki

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Abstract

The efficacy of azacitidine (AZA) on survival of lower risk (LR) - myelodysplastic syndromes (MDS) is controversial. To address this issue, we retrospectively evaluated the long-term survival benefit of AZA for patients with LR-MDS defined by International Prognostic Scoring System (IPSS). Using data from 489 patients with LR-MDS in Nagasaki, hematologic responses according to International Working Group 2006 and overall survival (OS) were compared among patients that received best supportive care (BSC), immunosuppressive therapy (IST), erythropoiesis-stimulating agents (ESA), and AZA. Patients treated with AZA showed complete remission (CR) rate at 11.3%, marrow CR at 1.9%, and any hematologic improvement at 34.0%, with

Abbreviations: AEs, adverse events; AML, acute myeloid leukemia; AZA, azacitidine; BSC, best supportive care; CI, comorbidity index; CI, confidence interval; CR, complete remission; ECOG, Eastern Cooperative Oncology Group; ESA, erythropoiesis-stimulating agents; FAB, French-American-British; HI, hematological improvement; HR, higher risk; HSCT, hematopoietic stem cell transplantation; int, intermediate; IPSS, International Prognostic Scoring System; IPSS-R, revised version; IST, immunosuppressive therapy; LR, lower risk; mCR, marrow CR; MDS, myelodysplastic syndromes; OR, Overall response; OS, overall survival; PC, platelet cells; PR, partial remission; PS, Performance status; RBC, red blood cells; TD, transfusion dependence; TI, transfusion independence; t-MDS, therapy-related MDS; WHO, World Health Organization.

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ORIGINAL ARTICLE

Nationwide epidemiological survey of familial myelodysplastic syndromes/acute myeloid leukemia in Japan: a multicenter retrospective study

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ABSTRACT

Although several pedigrees of familial myelodysplastic syndromes/acute myeloid leukemia (fMDS/AML) have been reported, the epidemiology and clinical features has been poorly understood. To explore the epidemiology of this entity, we performed a retrospective nationwide epidemiological survey in Japan using questionnaire sheets. The questionnaire was sent to 561 institutions or hospitals certified by Japanese Society of Hematology, unearthing the existence of 41 pedigrees of fMDS/AML. Among them, we obtained the clinical information of 31 patients in 20 pedigrees. The median age of the initial diagnosis was 51 years (range 9–88 years) and the WHO classification 2008 ranged from refractory anemia (RA) to AML. Focusing on the familial MDS patients, refractory anemia with excess blasts (RAEB)-2 was the largest group (27.3%). The median overall survival (OS) of fMDS and fAML in this study were 71.6 and 12.4 months, and the five-year OS were 61.3 and 50%, respectively.

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KEYWORDS
Myelodysplastic syndromes; familial MDS; familial AML; nationwide survey; retrospective study

Introduction

The myelodysplastic syndromes (MDS) are a group of clonal disorders of hematopoietic stem cells and are defined by ineffective hematopoiesis and/or bone marrow dysplasia [1–3]. More than 30,000 new cases of MDS occur in the US every year, and approximately 30% of MDS patients eventually progress to acute myeloid leukemia (AML) [4,5]. The median overall survival (OS) of MDS patients with International Prognostic Scoring System (IPSS) 'high' is around 0.4 years [6], thus, it is still challenging for physicians to treat severe MDS cases. Although allogeneic hematopoietic stem cell transplantation (HSCT) is still the only treatment to cure MDS, very limited MDS patients are considered an indication for HSCT due to the elderly [7]. Moreover, it is reported that five-year OS after allogeneic HSCT was 28% in the patients with refractory anemia with excess blasts (RAEB)-2 [8].

MDS or AML is mostly a sporadic disease, however, familial cases have been also recognized recently. Holme et al. [1] reported the large cohort of 27 families with familial MDS/AML (fMDS/AML) from the UK. Since many of the fMDS/AML cases have been recently defined, the guidelines for genetic analysis and clinical treatment are currently based on expert opinions [9]. In clinical settings, clinicians are required to take a detailed family history and consult with a certified genetic counselor when necessary [10]. The curative treatment for fMDS/AML is allogeneic HSCT only, same as the sporadic MDS [11]. Although epidemiology and disease characteristics of sporadic MDS/AML have been gradually uncovered, little is

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Supplemental data for this article can be accessed here.

第 82 回日本血液学会学術集会
骨髄系腫瘍：CML/MPN/MDS
25（EL2-3G）

輸血後鉄過剰症の診療

鈴木隆浩

難治性貧血疾患では赤血球輸血が必要とされるが，頻回の輸血は輸血後鉄過剰症のリスクとなる。過剰鉄は活性酸素種の産生を介して臓器障害を引き起こし，低リスク MDS では高フェリチン血症が予後不良因子の一つであることが知られている。このため，輸血後鉄過剰症では鉄キレート療法が行われる。輸血依存になった患者は，定期的に血清フェリチン値を確認し，フェリチン値 500 ng/ml 以上および総赤血球輸血量 20 単位以上で輸血後鉄過剰症と診断される。鉄キレート療法はフェリチン値 1,000 ng/ml 以上で開始し，有害事象などの問題がなければフェリチン値が 500 ng/ml 未満になるまで治療を継続する。鉄キレート療法によって臓器障害は改善し，低リスク MDS では臓器障害や死亡リスクの低減が期待される。一部症例では造血改善も認められるが，現時点では造血の改善を目的としたキレート療法は推奨されていない。（臨床血液 61（9）：1205～1211，2020）

Key words : Iron overload, Iron chelation therapy, Guideline

はじめに

再生不良性貧血や骨髄異形成症候群（myelodysplastic syndrome, MDS）などの難治性貧血疾患では赤血球輸血が行われるが，繰り返す赤血球輸血は鉄過剰症のリスクとなる。ヒトには積極的な鉄排泄機構が備わっておらず，出血や溶血がなければ鉄排泄量は 1 日に 1 mg 程度で一定である。わが国の赤血球輸血製剤は 1 単位あたり約 100 mg の鉄を含んでいることから，1 回 2 単位の輸血で 200 mg，つまり排泄に 200 日を要する鉄が体内に入ってくる計算になる。つまり，赤血球輸血を繰り返すと最終的に鉄過剰症に至ることになる。

過剰に蓄積した鉄は様々な臓器に沈着し，機能障害を引きおこす。鉄によって傷害されやすい臓器としては肝臓，心臓，内分泌腺が知られており，低リスク MDS では高フェリチン血症が独立した予後不良因子であることも明らかになってきた[1~3]。

このため，2010 年前後より各国で鉄過剰症におけるガイドライン作製が行われるようになり，わが国でも厚生労働科研「特発性造血障害に関する調査研究」班が主体となり 2008 年に「輸血後鉄過剰症の診療ガイド」が作製された[4]。同ガイドはわが国における初めての鉄過剰症治療指針であり，作製以来広く国内で利用されてきたが，初版作成から約 10 年が経過しその間に様々なエビデンスが蓄積されてきたため，2020 年に改訂第 2 版が発行された[5]。今回の改訂版より構成が変更されており，原発性鉄過剰症を含めた鉄過剰症全般についての教科書的総説と輸血後鉄過剰症については臨床上重要な項目について clinical question 形式での解説が記載されている。

本教育講演（本稿）では，改訂された診療ガイドの内容を中心に，輸血後鉄過剰症の病態，診断，治療について解説する。

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輸血後鉄過剰症の病態

鉄は生体内の酸化還元反応を担う重要な元素であり，その反応活性の高さからミトコンドリア内の呼吸鎖反応など様々な酸化還元に関わる反応に利用されている。しかし，その特性は生体にとって有害な酸化活性物質を産みやすいというリスクを同時にもたらすこととなり，鉄は生体にとって危険な存在でもある。鉄は生体内において，Fenton 反応や Haber-Weiss 反応などによって強い酸化能を持つ活性酸素種（reactive oxygen species, ROS）を産生する（Fig. 1）。その中でもヒドロキシラジカル（・OH）は最強の酸化能を持ち，脂質やタンパク，核酸の傷害を介して様々な細胞障害や臓器障害を引き起こすことが知られている。

このため生体内には反応性の高い鉄（遊離鉄）を抑え

特集
臨床血液学 —診断と治療におけるパラダイムシフト 2020（赤血球系疾患）—

鉄代謝と鉄剤不応性鉄欠乏性貧血

鈴木隆浩

体内鉄は様々な分子によって制御されているが，その中心的役割を担うのがヘプシジンである。鉄剤不応性鉄欠乏性貧血（iron refractory iron deficiency anemia, IRIDA）は，ヘプシジン抑制に関わるマトリプターゼ2（*TMPRSS6*遺伝子でコードされるセリンプロテアーゼ）の異常によって発症する。IRIDAでは出生時よりヘプシジン産生が亢進しているために腸管からの鉄吸収が障害され，鉄欠乏性貧血をきたすが，ヘプシジン高値のため血清フェリチン値は低下しない。腸管からの吸収低下のため経口鉄剤には不応であり，静注鉄剤にはある程度反応するのが特徴である。IRIDAは，ヘプシジン亢進が認められる慢性疾患に伴う貧血との鑑別が重要であり，診断には遺伝子検査が必須であるが，現状では検査困難であるため，その疫学や詳細な臨床像は十分に分かっていない。（臨床血液 61（5）：475～483，2020）

Key words : Iron refractory iron deficiency anemia (IRIDA), Hepcidin, *TMPRSS6* gene, Hemojuvelin (HJV)

はじめに

鉄欠乏性貧血は体内鉄総量の絶対的減少によってヘモグロビン合成が低下し，その結果発症する貧血である。通常小球性低色素性貧血を呈し，単一の貧血症としては最も頻度が高い。鉄欠乏性貧血は，ほとんどの場合鉄剤投与および鉄欠乏をきたす原因疾患の治療で改善させることができるが，時に難治性の症例を経験する。その原因は様々であり，鉄の吸収障害，鉄以外の造血因子・ビタミンの不足などを考慮する必要があるが（Table 1）（詳細は筆者が2016年に本誌に投稿した総説[1]を御覧いただければ幸いである），最近その一部に遺伝性疾患が存在することが明らかになってきた。本稿では，「鉄剤不応性鉄欠乏性貧血（iron refractory iron deficiency anemia, IRIDA）」と名付けられた遺伝性鉄欠乏性貧血について述べる。IRIDAは鉄制御ペプチドホルモンであるヘプシジンの過剰産生を本態とする鉄欠乏性貧血であるため，鉄代謝メカニズムについても頁を割いて解説したい。

鉄の吸収メカニズム

生理的条件下で鉄は全て食物摂取を通じて体内に取り込まれ，十二指腸・上部空腸で吸収される。食餌中の鉄はヘム鉄と非ヘム鉄の二種類の形で存在し，前者は主に動物性食餌に含まれ，後者は植物性食餌に含まれるが，これらは体内での吸収様式が異なる[2]。

ヘム鉄はFe^{2+}とポルフィリンからなる錯体であるが，胃液によって消化され消化液中に放出された後，ヘムは水溶性のまま上部小腸に到達し，小腸上皮細胞上のヘム輸送蛋白（heme-carrier protein 1（HCP1），または proton-coupled folate transporter（PCFT）とも呼ばれる）を介して上皮細胞内に吸収されると考えられている（吸収の詳細は現在でも明確になっておらず，そのメカニズムについては依然議論が続いている）。腸管上皮細胞内に入ったヘムはヘム酸素添加酵素（heme oxygenase 1, HO）によって分解され，Fe^{2+}が取り出される。

一方，食餌中の非ヘム鉄は主に3価鉄（Fe^{3+}）で存在するが，Fe^{3+}は中性・アルカリ性液中で難溶性となるためそのままでは吸収が困難である。Fe^{3+}は強酸中では可溶化されることから，非ヘム鉄は胃酸によって消化された後，消化液中に溶解し，腸液によるアルカリ化で一部難溶化しつつも上部小腸で腸管上皮細胞に吸収される（Fig. 1）。吸収の際，Fe^{3+}は腸管上皮細胞上に発現する十二指腸チトクロームB（duodenal cytochrome b, DcytB）などによって2価（Fe^{2+}）に還元され，2価金属輸送体（divalent metal transporter 1, DMT1）を通して上皮細胞内に取り込まれる。

腸管上皮細胞内に入ったFe^{2+}は細胞内の鉄担体であるポリrC結合蛋白（poly rC-binding protein, PCBP）に

北里大学医学部　血液内科学

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ORIGINAL ARTICLE

Treatment outcomes of chronic-phase chronic myeloid leukemia with resistance and/or intolerance to a 1st-line tyrosine kinase inhibitor in Japan: the results of the New TARGET study 2nd-line

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Abstract

We herein report the results of the New TARGET study 2nd-line, which collected data on patients with chronic-phase (CP) chronic myeloid leukemia (CML) who received a 2nd-line tyrosine kinase inhibitor (TKI) because of resistance and/or to a 1st-line TKI. A total of 98 patients were enrolled intolerance between April 2010 and March 2013, and 82 patients were analyzed. The median age was 54 years (range 22–88 years). Seventy-six patients (93%) received imatinib as the 1st-line TKI. Forty-five (55%) and 37 (45%) patients began nilotinib and dasatinib treatments at entry, respectively. First-line TKI treatment achieved complete hematological response in 79 patients (96%) and complete cytogenetic response (CCyR) in 49 patients (60%), respectively. Nine patients (11%) had *BCR-ABL1* kinase domain point mutations at enrollment. The estimated 3-year progression-free-survival rate after enrollment was 98.7% (95% CI 91.1–99.8%). Overall, the probabilities of achieving CCyR and a major molecular response were 89.3% (95% CI 81.4–94.8%) and 87.2% (95% CI 78.1–93.8%), respectively. There were no new safety issues. This study demonstrated that CML-CP patients in Japan who are resistant and/or intolerant to a 1st-line TKI can achieve an extremely good outcome by 2nd-line TKI treatment.

Keywords Chronic myeloid leukemia · Resistance or intolerance · Tyrosine kinase inhibitors · Observational study · Japan

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ORIGINAL ARTICLE

Long-term outcome in patients with Fanconi anemia who received hematopoietic stem cell transplantation: a retrospective nationwide analysis

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Abstract

We retrospectively analyzed nationwide records of 163 Fanconi anemia (FA) patients [aplastic anemia (AA), $n=118$; myelodysplastic syndrome (MDS), $n=30$; acute leukemia, $n=15$] who underwent first allogeneic hematopoietic stem cell transplantation (HSCT) between 1987 and 2015 in Japan. An alternative donor was used in 119 (73%) patients, and 160 (98%) patients received a non-T-cell-depleted graft. With an 8.7-year median follow-up, 5-year overall survival (OS) was 81%. The 5-year OS was significantly higher in AA patients than in MDS and acute leukemia patients (89%, 71%, and 44%, respectively). In the MDS/leukemia group, factors associated with poor outcome in univariate analysis were older age at HSCT (≥ 18 years), conditioning regimen without anti-thymocyte or lymphocyte globulin, and grade II–IV acute graft-versus-host disease. After 1 year, of 137 survivors, 15 developed subsequent malignancies, of whom 12 were diagnosed with head and neck (HN)/esophageal cancer. An irradiation regimen and older age were associated with the risk of HN/esophageal cancer. Five of seven deaths were attributed to subsequent malignancies more than 5 years after HSCT. On the basis of the risk factors for HSCT in MDS/leukemia patients and subsequent malignancies, a more effective HSCT approach is required.

Keywords Fanconi anemia · Hematopoietic stem cell transplantation · Myelodysplastic syndrome · Acute leukemia · Subsequent malignancies after hematopoietic stem cell transplantation

Introduction

Fanconi anemia (FA) is a rare genetic disorder characterized by malformations, progressive bone marrow failure, and a predisposition to developing malignancy, mainly myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) and head and neck (HN) squamous cell carcinoma (SCC) [1]. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for hematological disorders in FA patients. Its conditioning regimen has been modified by reducing the cyclophosphamide (CY) and radiation doses [2], by fludarabine (FLU) administration [3], and by T-cell-depleted marrow allografts [4]. These result in a significant improvement of survival in FA patients. However, data on HSCT in FA patients affected by MDS/acute leukemia are scarce [5]. We present the characteristics and results of HSCT in FA patients in Japan and compare them with those of aplastic anemia (AA) and MDS/acute leukemia patients. Additionally, we evaluate the incidence and risk factors of subsequent malignancies in Japanese FA patients by comparing them with those in previous reports.

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RESEARCH ARTICLE

Clinical features of children with polycythemia vera, essential thrombocythemia, and primary myelofibrosis in Japan: A retrospective nationwide survey

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Abstract

Background: Philadelphia-negative (Ph-negative) myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are exceptionally rare during childhood. Thus, clinical

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ORIGINAL ARTICLE

Nationwide epidemiological survey of familial myelodysplastic syndromes/acute myeloid leukemia in Japan: a multicenter retrospective study

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ABSTRACT

Although several pedigrees of familial myelodysplastic syndromes/acute myeloid leukemia (fMDS/AML) have been reported, the epidemiology and clinical features has been poorly understood. To explore the epidemiology of this entity, we performed a retrospective nationwide epidemiological survey in Japan using questionnaire sheets. The questionnaire was sent to 561 institutions or hospitals certified by Japanese Society of Hematology, unearthing the existence of 41 pedigrees of fMDS/AML. Among them, we obtained the clinical information of 31 patients in 20 pedigrees. The median age of the initial diagnosis was 51 years (range 9–88 years) and the WHO classification 2008 ranged from refractory anemia (RA) to AML. Focusing on the familial MDS patients, refractory anemia with excess blasts (RAEB)-2 was the largest group (27.3%). The median overall survival (OS) of fMDS and fAML in this study were 71.6 and 12.4 months, and the five-year OS were 61.3 and 50%, respectively.

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KEYWORDS
Myelodysplastic syndromes; familial MDS; familial AML; nationwide survey; retrospective study

Introduction

The myelodysplastic syndromes (MDS) are a group of clonal disorders of hematopoietic stem cells and are defined by ineffective hematopoiesis and/or bone marrow dysplasia [1–3]. More than 30,000 new cases of MDS occur in the US every year, and approximately 30% of MDS patients eventually progress to acute myeloid leukemia (AML) [4,5]. The median overall survival (OS) of MDS patients with International Prognostic Scoring System (IPSS) 'high' is around 0.4 years [6], thus, it is still challenging for physicians to treat severe MDS cases. Although allogeneic hematopoietic stem cell transplantation (HSCT) is still the only treatment to cure MDS, very limited MDS patients are considered an indication for HSCT due to the elderly [7]. Moreover, it is reported that five-year OS after allogeneic HSCT was 28% in the patients with refractory anemia with excess blasts (RAEB)-2 [8].

MDS or AML is mostly a sporadic disease, however, familial cases have been also recognized recently. Holme et al. [1] reported the large cohort of 27 families with familial MDS/AML (fMDS/AML) from the UK. Since many of the fMDS/AML cases have been recently defined, the guidelines for genetic analysis and clinical treatment are currently based on expert opinions [9]. In clinical settings, clinicians are required to take a detailed family history and consult with a certified genetic counselor when necessary [10]. The curative treatment for fMDS/AML is allogeneic HSCT only, same as the sporadic MDS [11]. Although epidemiology and disease characteristics of sporadic MDS/AML have been gradually uncovered, little is

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Supplemental data for this article can be accessed here.

JCI insight

RESEARCH ARTICLE

MAPK mutations and cigarette smoke promote the pathogenesis of pulmonary Langerhans cell histiocytosis

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Pulmonary Langerhans cell histiocytosis (PLCH) is a rare smoking-related lung disease characterized by dendritic cell (DC) accumulation, bronchiolocentric nodule formation, and cystic lung remodeling. Approximately 50% of patients with PLCH harbor somatic *BRAF-V600E* mutations in cells of the myeloid/monocyte lineage. However, the rarity of the disease and lack of animal models have impeded the study of PLCH pathogenesis. Here, we establish a cigarette smoke–exposed (CS-exposed) BRAF-V600E–mutant mouse model that recapitulates many hallmark characteristics of PLCH. We show that CD11c-targeted expression of BRAF-V600E increases DC responsiveness to stimuli, including the chemokine CCL20, and that mutant cell accumulation in the lungs of CS-exposed mice is due to both increased cellular viability and enhanced recruitment. Moreover, we report that the chemokine CCL7 is secreted from DCs and human peripheral blood monocytes in a BRAF-V600E–dependent manner, suggesting a possible mechanism for recruitment of cells known to dominate PLCH lesions. Inflammatory lesions and airspace dilation in BRAF-V600E mice in response to CS are attenuated by transitioning animals to filtered air and treatment with a BRAF-V600E inhibitor, PLX4720. Collectively, this model provides mechanistic insights into the role of myelomonocytic cells and the BRAF-V600E mutation and CS exposure in PLCH pathogenesis and provides a platform to develop biomarkers and therapeutic targets.

Introduction

Pulmonary Langerhans cell histiocytosis (PLCH) is a rare smoking-related interstitial lung disease characterized by bronchiolocentric, Langerin$^+$ dendritic cell (DC) accumulation and cystic remodeling of the lung. PLCH typically manifests as a single-system disorder with pulmonary impairment ranging from asymptomatic disease to life-threatening respiratory failure (1) and is pathophysiologically distinct from the LCH of children that often results in visceral infiltration, bone lesions, and multisystem involvement (2). PLCH in adults occurs almost exclusively in active smokers, with an estimated prevalence of 0.27 and 0.07 per 100,000 population in males and females, respectively, and represents up to 5% of the interstitial lung diseases seen in referral centers (3, 4). Little is known about the pathogenesis of PLCH, which was long felt to be a reactive scarring process driven by tobacco use. The discovery over 2 decades ago of recurrent PLCH in the allografts of patients with PLCH who had undergone lung transplantation, however, suggested a metastatic mechanism (5). The recent identification of several activating mutations in the mitogen-activated protein kinase (MAPK) pathway in more than 70% of patients with PLCH (6, 7) is consistent with a metastatic model and suggests that PLCH may be most appropriately classified as an inflammatory neoplastic disorder (8, 9). The most common mutation identified to date has been a V600E

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●症 例

ネオシーダーによって再燃した肺ランゲルハンス細胞組織球症の1例

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要旨：51歳，女性．胸部単純X線検査で両側肺野に一部空洞を伴う多発結節影を認め，当院紹介受診となった．胸腔鏡下手術による肺生検と全身精査により肺ランゲルハンス細胞組織球症と診断した．禁煙により多発結節影が軽減したが，ネオシーダーの吸入により多発結節影が再燃した．ネオシーダーの吸入を中止したところ，肺野の多発結節影は再度軽減した．ネオシーダーが喫煙関連疾患を悪化させた報告はこれまで認められていない．紙巻きタバコと同成分が含まれているという報告があり，共通の成分が肺ランゲルハンス細胞組織球症の増悪に関与していると推測された．

キーワード：ランゲルハンス細胞組織球症，喫煙，ネオシーダー
Langerhans-cell histiocytosis (LCH), Smoking, NEO CEDAR

緒 言

肺ランゲルハンス細胞組織球症（pulmonary Langerhans-cell histiocytosis：PLCH）は主に若年成人に発症する嚢胞性間質性肺疾患であり，喫煙に関連している．今回禁煙に成功して改善したPLCHの患者が，指定第2類医薬品であるネオシーダーによって再燃した症例を経験した．ネオシーダーによる再燃報告はなく，貴重な症例であると考えられたため報告する．

症 例

患者：51歳，女性．
主訴：倦怠感．
既往歴：虫垂炎．
内服歴：薬剤・サプリメント・漢方薬なし．
家族歴：父 大腸癌，母 大腸癌，叔父 肺癌．
喫煙歴：23歳～現在，5本/日（紙巻きタバコ）．
飲酒歴：ビール350mL/日．
仕事歴：看護師．
現病歴：20XX年1月から仕事が多忙になり，倦怠感が増悪し，市販の感冒薬を服用して様子をみていた．20XX年8月下旬に近医で血液検査と胃透視検査を施行したが異常は認めなかった．胸部単純X線検査で両側に多発する結節影を認めたため，前医を受診した．前医で気管支鏡検査を行ったが，確定診断には至らず精査と胸腔鏡（video-assisted thoracic surgery：VATS）下肺生検目的に当院を紹介受診，入院となった．

初診時現症：体温36.5℃，血圧142/94mmHg，脈拍65/min・整，呼吸数24/min，SpO_2 97%（室内気），眼瞼結膜蒼白なし，眼球結膜黄染なし，咽頭発赤・疼痛・扁桃腫大なし，呼吸音正常，倦怠感あり，心音正常．腹部平坦・軟，圧痛なし．四肢浮腫なし，皮疹なし．

入院時血液検査所見（表1）：末梢血液検査では，白血球数7,600/μL，CRP 0.47mg/dL，抗MAC抗体陰性，クオンティフェロン陰性だった．また，CEA 3.3ng/mL，CYFRA 1.0ng/mLと正常範囲内であった．

画像所見：胸部単純X線写真では両側に多発する小結節影を認めた（図1A）．胸部HRCTでも上葉優位に両側肺野に多発性に結節，空洞を伴う結節，気管支周囲結節が認められた（図2B）．有意なリンパ節腫大はみられなかった．PET-CTでも胸部HRCTと同様な結節影と空洞形成を認め，FDG-PETにて，結節に相当する異常集積［maximum standardized uptake value (SUVmax) = 3.5］を認めた．肺以外の領域に悪性病変を示唆する異常集積は認めなかった（図2A）．

臨床経過：気管支鏡を再度勧めたが本人が同意せず，外科でVATSを行う方針となった．転移性肺腫瘍や肺癌の多発肺転移や結核などを疑って採血，PET-CTを行っ

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