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CASE REPORT

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Bone marrow particle enrichment analysis for the laboratory diagnosis of multiple myeloma: A case study

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Abstract

Background: Bone marrow smear and biopsy are the main methods for the diagnosis of multiple myeloma (MM), bone marrow infiltration, and metastasis in lymphoma and cancer. However, several factors, including the focal growth of tumor cells, inappropriate puncture sites, and hemodilution of bone marrow aspirates, lower the rate of target cell detection. To solve this problem, we developed a novel method—bone marrow particle enrichment analysis—and here, we describe this procedure and its use in the diagnosis of a rare case of MM.

Methods: An 88-year-old man with primary gastric gamma delta T-cell lymphoma (γδTCL) was found to have anemia. As the cause of anemia could not be determined, hemodilution was suspected, warranting the re-examination of the bone marrow aspirate. Re-puncture could not be performed because of the patient's age and unwillingness to undergo this procedure. Hence, we used a novel approach to enrich bone marrow particles and isolate marrow cells, and subsequently performed morphological and flow cytometric analysis.

Results: Examinations performed after bone marrow particle enrichment revealed the presence of myeloma cells, and the patient was diagnosed with primary gastric γδTCL accompanied by MM.

Conclusions: Bone marrow particle enrichment analysis may be applied to overcome the problems caused by hemodilution of bone marrow aspirates and to improve the rate of tumor cell detection. The application of this method for the diagnosis of hematological disorders should be explored further.

KEYWORDS

bone marrow particles, diagnosis, enrichment analysis, gastric γδT-cell lymphoma, multiple myeloma

Congming Zhang and Yanan Zhang contributed equally to this work.

1 | INTRODUCTION

Bone marrow aspiration and biopsy are the main methods for the diagnosis of multiple myeloma, lymphoma with bone marrow infiltration, and cancer with bone marrow metastasis.[1] Hemodilution of bone marrow aspirates is the main contributor to a missed diagnosis after the examination of bone marrow smears. Moreover, the location of the puncture site and the degree of cancer cell dispersion also affect the rate of positive findings on bone marrow biopsy, as the growth of myeloma cells is localized, and tumor cells from lymphoma and cancer in the bone marrow are limited.[2]

In the absence of sufficient evidence to convince patients who are not willing to undergo multiple punctures for bone marrow aspiration or biopsy, re-analysis of previously diluted bone marrow aspirates becomes necessary. In order to overcome these challenges and ensure the adequate detection of target cells in bone marrow, bone marrow aspirates need to be optimized. To solve this problem, we developed a novel method—bone marrow particle enrichment analysis—and here, we describe this procedure and its use in the diagnosis of a rare case of MM.

2 | CASE PRESENTATION

An 88-year-old man with abdominal distention was hospitalized in the gastrointestinal surgery department. He had a past history of schistosoma when he was a teenager, but had no past history of tuberculosis, chronic bronchitis, or tumors and had no special medication history. No obvious bone pain, cough, shortness of breath, hematochezia, or other type of discomfort was reported. Enhanced abdominal computed tomography revealed an abnormal enhanced focus in the lower gastric body, antrum, and pylorus (Figure 1A), and splenomegaly was also observed. A giant gastric ulcer was found on gastroscopy (Figure 1B), and endoscopic biopsy and immunohistochemistry revealed the presence of lymphoma cells expressing CD45RO as well as positivity for T-cell differentiation markers (CD3, CD56, and TIA-1; Figure 1C). TCR gamma delta clonal gene rearrangement was also detected (Figure 1D). Based on this as well as clinical observations, the patient was diagnosed with primary gastric gamma delta T-cell lymphoma (γδTCL)[3] and transferred to the hematology department for further diagnosis and treatment.

Subsequently, bone marrow aspiration and smear were performed for lymphoma staging. Plasma cells accounted for 2% of the total cell count, and no lymphoma cells or abnormal plasma cells were observed (Figure 2A). However, routine blood examination revealed the presence of anemia (hemoglobin level, 77 g/L) that had occurred before lymphoma-related symptoms appeared. In view of the normal mean corpuscular volume(MCV), negative results on the fecal occult blood test, normal levels of ferritin, and adequate proliferation in bone marrow, the cause of anemia remained unknown. Therefore, bone marrow aspirates were clinically presumed to have been diluted by blood. However, re-puncture was challenging owing to the patient's advanced age and his unwillingness to undergo this procedure. Hence, we optimized the previous bone marrow aspirates.

We centrifuged 4 mL of the previously acquired bone marrow aspirate that had been preserved in an anticoagulant tube containing ethylenediamine tetraacetic acid at 100 *g* for 5 minutes and then pipetted the mixture of the upper bone marrow particles and plasma into 1.5-mL EP tube using a Pasteur pipette. A syringe with a 25G stainless steel flat-mouthed hollow needle was inserted into the mixture and aspirated thrice to create a suspension of bone marrow particles. After centrifugation for 8 minutes at 1500 *g*, the supernatant above the plasma was removed, the remaining plasma was mixed with the cells at the bottom, and then the residual fiber components were filtered out using a 300-mesh nylon filter net. As a result, bone marrow particle cells were enriched in the collected filtrate, which was then used for bone marrow smear and flow cytometry analysis. The detailed procedure used for bone marrow particle enrichment analysis is shown in Figure S1A-D. The layer floating above the plasma was crushed into a film, revealing isolated bone marrow particles (Figure S2).In the smear containing enriched bone marrow cells, plasma cells accounted for 11.0% of the cell count, and some cells had obvious morphologic anomalies(Figure 2B). Flow cytometry analysis performed using the enriched bone marrow particles revealed that the abnormal plasma cell populations expressed CD138, CD38, and CD56, and accounted for 12.49% of all cells (Figure 2C).

To further confirm these findings, bone marrow biopsy and monoclonal M protein detection were performed. Scattered abnormal plasma cells were observed in the bone marrow biopsy, and these cells strongly expressed plasmacytoma differentiation markers (CD138, CD38, and CD56). Monotypic lambda light chain restrictive expression was also observed, and the pathological diagnosis was thus plasmacytoma (Figure 2D). The monoclonal M protein detected using serum immunofixation electrophoresis was IgG-lambda (Figure 2E), and a quantitative assay showed that IgG levels were within the normal range (10 g/L), while IgA and IgM levels were significantly below normal. The serum lambda light chain levels were abnormally high at 5.2 g/L, while kappa light chain levels were significantly below normal at 0.55 g/L. Additionally, the serum creatinine level was 112 μmol/L, indicating renal insufficiency, although it did not exceed 177 μmol/L. Finally, based on the combination of the proportion of bone marrow plasma cells and bone marrow biopsy results, monoclonal M protein findings, and the presence of renal insufficiency and myeloma-related anemia, the patient was diagnosed with MM.[4]

Chemotherapy with cytotoxic drugs was contraindicated because the patient had emphysema, pulmonary bulla, and multiple cysts in both kidneys, and the comprehensive geriatric assessment revealed that he was weak. Thus, he was given lenalidomide for targeted therapy and ubenimex for adjuvant therapy, with stomach care and nutritional support. The patient was discharged after hospitalization for 24 days. The treatment was found to be effective

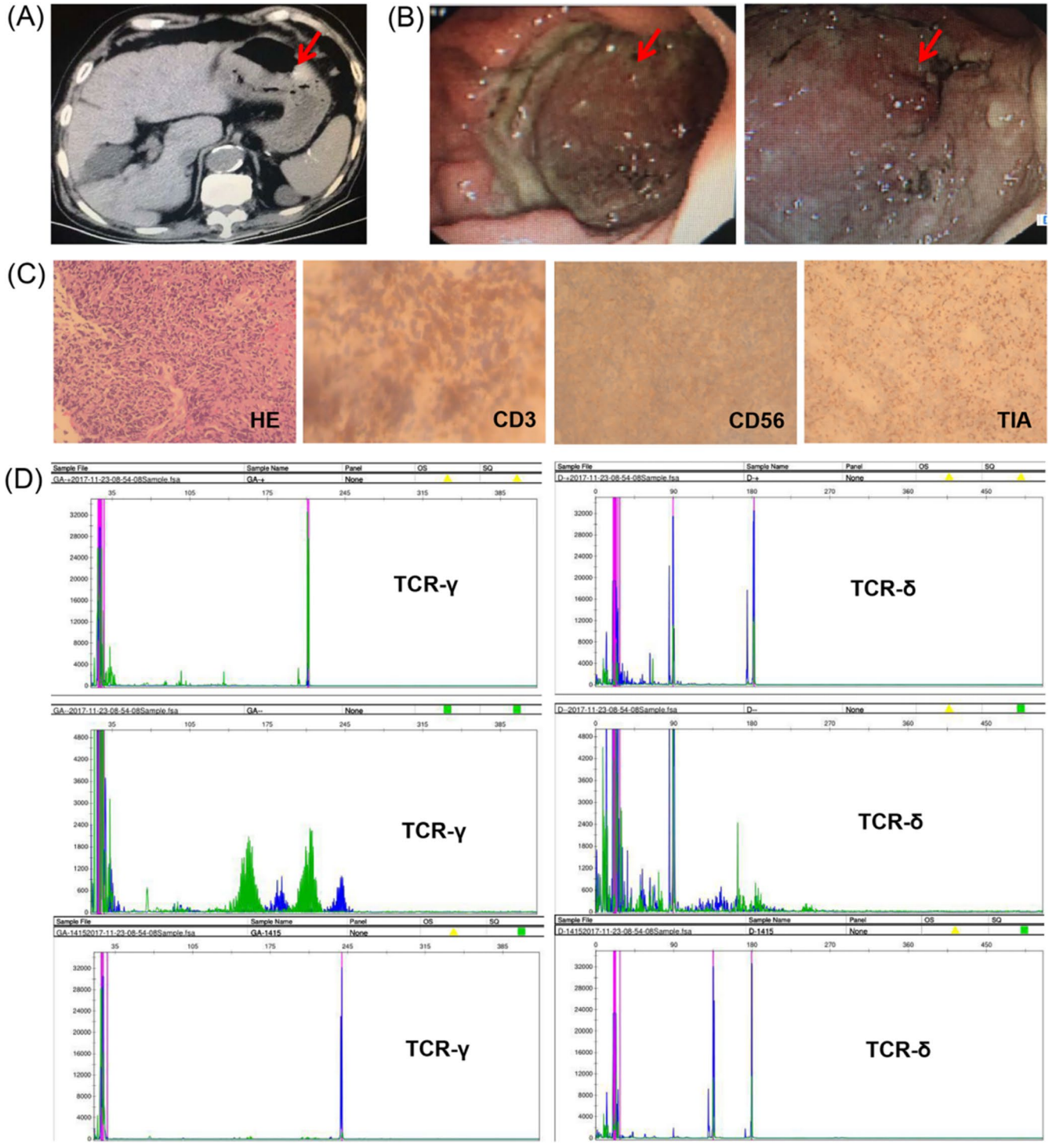

FIGURE 1 Diagnosis of primary gastric γδTCL. A, Abnormal enhanced focus observed in the stomach on an enhanced computed tomography scan (red arrow). B, Giant gastric ulcer revealed on endoscopy. C, Lymphoma cells observed on gastric biopsy and immunohistochemical analysis(HE, ×400; CD3, ×400; CD56, ×400; TIA, ×400). D, Image showing TCR gamma delta clonal gene rearrangement

on subsequent follow-up assessment by a telephonic interview on the 14th day after discharge. With thalidomide, low-dose dexamethasone, and ubenimex as an intermittent treatment.[5] Although the patient was stable for 41 days after discharge, he unfortunately died suddenly for unknown reasons.

3 | DISCUSSION

γδTCL is a rare subtype of peripheral T-cell lymphoma. Primary liver, spleen, and skin γδTCL are the main forms of γδTCL commonly reported in the literature,[6] but primary gastric γδTCL is rare. Our

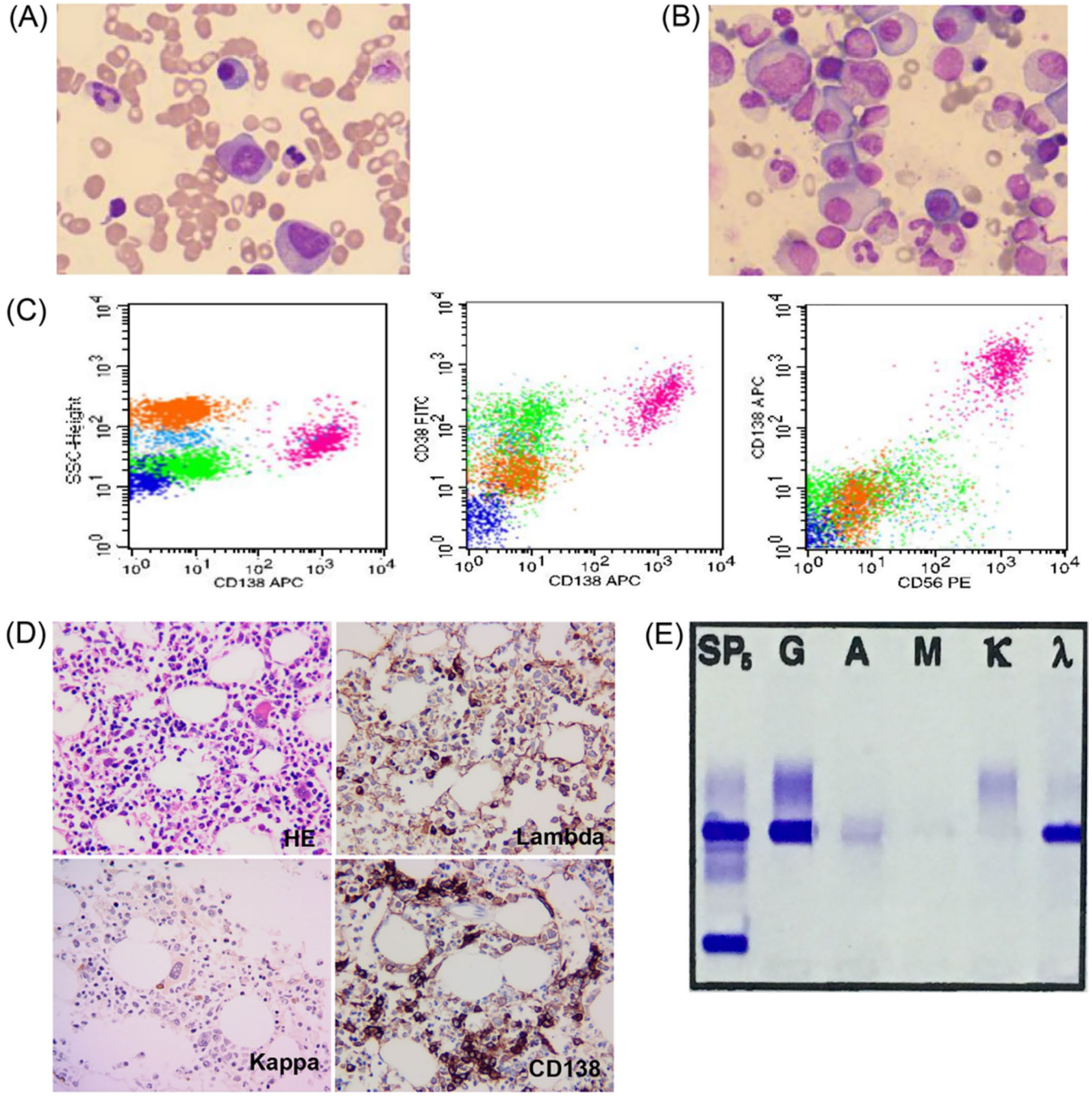

FIGURE 2 Diagnosis of multiple myeloma. A, Plasma cells, accounting for 2% of all cells, with no abnormal plasma cells or lymphoma cells in the bone marrow smear (Wright-Giemsa, ×1000). B, Plasma cells, accounting for 11.0% of all cells, after bone marrow particle enrichment (Wright-Giemsa, ×1000). C, Abnormal plasma cells in bone marrow particles, accounting for 12.49% of all cells, showing $CD138^+$, $CD38^+$, and $CD56^+$ immunophenotypes on flow cytometry analysis after enrichment. D, Scattered or piles of abnormal plasma cells observed on pathological analysis of the bone marrow biopsy (HE, ×400; Lambda, ×400; Kappa, ×400; CD138, ×400). E, Monoclonal M protein (IgG-lambda type) observed using serum immunofixation electrophoresis

findings suggest that primary gastric γδTCL and MM can occur simultaneously as two independent hematological malignancies. Although γδTCL is a highly aggressive neoplasm with a poor prognosis and there is no effective standard treatment regimen,[3] lenalidomide-targeted therapy for MM was effective in this patient. Lenalidomide is mainly used in MM therapy, but it has been reported to be effective in the treatment of peripheral T-cell lymphoma.[7] Therefore, the patient in this case was treated with lenalidomide which might be effective for γδTCL as well.

Bone marrow examination is necessary for newly diagnosed lymphoma patients.[8] Bone marrow aspiration is the most commonly used procedure for such examinations in our country, whereas bone marrow biopsy is not routinely used due to its high cost and the sophisticated equipment required. In the present case, the patient had

already been diagnosed with gastric γδTCL, and routine examination of the bone marrow aspiration smear was performed to assess the clinical stage of lymphoma. However, the results of the bone marrow smear could not explain the severe anemia observed, and there was no evidence of hemorrhagic, iron deficiency, or renal anemia. Hence, we suspected that the bone marrow aspirates may have been diluted and that the results were not accurate. Therefore, we developed a novel method—bone marrow particle enrichment analysis—and described the specific process and implementation of this method in this case.

Bone marrow particle enrichment analysis is simple and easy to perform. The samples used are conventional bone marrow aspirates. In general, bone marrow particles can be obtained from most patients using standard bone marrow aspiration. Moreover, stainless steel flat-mouthed hollow needles, EP tubes, and Pasteur pipettes are also easy to obtain, and the entire process can be completed in half an hour. Using this method, in this case, abnormal plasma cells were discovered and flow cytology, bone marrow biopsy, and serum immunofixation electrophoresis were subsequently performed to confirm multiple myeloma.

Multiple punctures may have adverse effects. Therefore, several cases of bone marrow metastasis of cancer and plasma cell disease wherein bone marrow smears and biopsy were unreliable because aspirate was drawn from a single site have been diagnosed using this method in the past year. In 2008, the International Committee for Standardization in Hematology recommended that particle crush slide preparations should be used for the microscopic evaluation of bone marrow.[9] However, the crush film only reveals morphology. In this case, bone marrow particle enrichment analysis could be used for morphological and immunological analysis.

Bone marrow particle enrichment analysis could have other applications too. In our laboratory, bone marrow particle enrichment analysis has also been used for detecting an *AML1-ETO* fusion gene using PCR in a leukemia patient in order to detect minimal residual disease, and we found that this method has better sensitivity than does standard marrow aspiration. This fusion gene was detected in this case; however, it was not detected before the bone marrow aspirate was optimized.

4 | CONCLUSION

Bone marrow particle enrichment analysis is a simple and practical method. It was applied clinically in this case, and the diagnosis of MM was not missed. This method uses specimens obtained directly from the hematopoietic parenchyma, and its clinical value in laboratory diagnosis for hematological disorders should be explored further.

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AUTHOR CONTRIBUTIONS

Congming Zhang designed the experimental method, performed the morphology test, and wrote the first draft. Yanan Zhang performed the flow cytometry test and revised the draft. All authors read and approved the final manuscript.

ETHICAL APPROVAL

Informed consent has been obtained by the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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