Clin. Lab. 2024;70:XXX-XXX

CASE REPORT

One Case of Abnormal Decrease of Interferon Gamma Release Assay Result Caused by Melphalan

Gangfeng Li, Tao Lu, Ningping Shan

Department of Clinical Laboratory Center, Shaoxing People's Hospital, Shaoxing, P.R. China

SUMMARY

Background: **Interferon gamma release assay (IGRA) is an important method to detect the specific antigen of tuberculosis, which is crucial to the diagnosis of tuberculosis or potential tuberculosis infection.**
Methods: **We report a case of myelosuppression caused by the use of Melphalan in the treatment of multiple myeloma, resulting in an abnormal decrease in interferon gamma release assay results.**
Results: **We collected blood samples from the patient for retesting and the result of the test did not differ significantly. Upon reviewing the case, it was found that the patient's use of Melphalan treatment resulted in bone marrow suppression and extreme reduction of peripheral blood lymphocytes. Therefore, it is speculated that the abnormal decrease of the interferon gamma release assay result is caused by bone marrow suppression, which is caused by the use of Melphalan.**
Conclusions: **When patients with multiple myeloma are treated with Melphalan, it can lead to bone marrow suppression and result in false negative interference gamma release assay results. Laboratory staff should consider the existence of such interference and communicate with clinical doctors in a timely manner.**
(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240437)

Correspondence:
Ningping Shan
Department of Clinical Laboratory Center
Shaoxing People's Hospital
Shaoxing 312000
P.R. China
Phone: +86 13957558155
Email: 553897163@qq.com

KEYWORDS

Melphalan, bone marrow suppression, interferon gamma release assay

INTRODUCTION

Interferon gamma release assay is a quantitative detection of interferon gamma released by peripheral blood memory T lymphocytes under the stimulation of tuberculosis specific antigens. It is often used for auxiliary diagnosis of tuberculosis [1]. We found a case of multiple myeloma in a patient who experienced immunosuppression due to treatment with Melphalan, resulting in false negative results for the interferon gamma release assay. The specific situation is as follows.

CASE PRESENTATION

The patient was a 63-year-old female. On February 1, 2023, the patient was admitted due to multiple myelo-

Case Report accepted May 2, 2024

ma. Admission examination: WBC 3.76 x 10^9/L; Immunoglobulin G 2.29 g/L ↓, immunoglobulin A 0.31 g/L ↓, total protein 50.8 g/L ↓, albumin 37.2 g/L ↓, globulin 13.6 g/L ↓. There are no significant abnormalities in urine routine, serum troponin, coagulation spectrum, fecal routine, etc. Chest CT: Scattered exudative lesions in both lungs with bilateral pleural reactions. On February 10, 2023, the result of the interferon gamma release assay was 802.4 pg/mL (reference value: 0 - 20 pg/mL), far exceeding the reference range. The potential of tuberculosis infection was considered and preventive antituberculosis treatment was done for the patient. On February 24, 2023, the interferon gamma release assay was retested and the result was 3.94 pg/mL (Table 1).

Due to the sharp decline in the result of the interferon gamma release assay compared to last time, clinical doctors have raised doubts about the result and contacted our department. With this question in mind, the laboratory staff first checked the status of the instruments and reagents on that day and checked the indoor quality control, which was completely under control. The possibility of detection errors caused by instrument and reagent abnormalities has been ruled out. At the same time, we reextracted blood from the patient for retesting, and the result was 3.87 pg/mL. Therefore, we reviewed the patient's case data and found that the patient started using Melphalan chemotherapy on February 17th, which resulted in bone marrow suppression. Both the day before and the day after the interferon gamma release assay, blood routine tests showed extreme reduction in lymphocytes. On February 23rd, lymphocytes were not even detected, at 0 x 10^9/L (Table 2). We speculate that Melphalan causes bone marrow suppression in patients, leading to a sharp decrease in peripheral blood lymphocytes. The sharp decrease in peripheral blood lymphocytes cannot produce sufficient response to the stimulation of tuberculosis specific antigens, leading to false negative test results. We suggest that doctors wait for the patient's bone marrow suppression to subside before conducting interferon gamma release assay. After the patient's peripheral lymphocytes returned to normal levels, we conducted an interferon gamma assay retest on March 17th. The results showed that 168.5 pg/mL returned to positive.

DISCUSSION

Tuberculosis is a chronic infectious disease caused by mycobacterium tuberculosis, which can affect all organs of the body and seriously endanger human health. Among the existing diagnostic methods, bacteriological examination is the gold standard for the diagnosis of tuberculosis, but its sensitivity is low. The tuberculin test (TST) is susceptible to immune status and has a cross reaction with BCG vaccination, leading to false positive results [2]. Interferon gamma release assay, as an *in vitro* diagnostic technique based on cellular immunity, detects interferon gamma secreted by memory T lymphocytes in fresh human peripheral blood upon reexposure to Mycobacterium tuberculosis specific antigen stimulation. According to the level of interferon gamma, judge whether it has T lymphocyte specific reaction against Mycobacterium tuberculosis, which is used for auxiliary diagnosis of tuberculosis [3]. This method is not affected by BCG vaccination and the interference of the vast majority of non tuberculosis mycobacteria, and is widely used in clinical practice.

The level of interferon gamma in this patient dropped sharply in a short time. Clinicians considered that tuberculosis could not be cured in a short time, and expressed doubts about this result. So the laboratory personnel conducted an inspection of the testing system and did not find any problems. At the same time, the patient's blood was reextracted for retesting, and the difference was not significant. So we looked up the patient's medical records and found that the patient started using Melphalan for treatment one week before the test. The day before the test, the patient's blood routine showed WBC 1.5 x 10^9/L, lymphocyte 0 x 10^9/L with grade 3 bone marrow suppression. The day after the test, the patient's blood routine showed WBC 0.02 x 10^9/L, lymphocyte 0.01 x 10^9/L with grade 4 bone marrow suppression. Melphalan is a peptide conjugated alkylating drug for the treatment of multiple myeloma, which exerts anti-tumor activity through DNA cross-linking [4]. Bone marrow suppression is one of the common side effects of Melphalan chemotherapy, usually seen 1 - 3 weeks after chemotherapy, with a predominant decrease in white blood cells [5]. Research has reported that patients with very low CD4 T lymphocyte counts have an adverse effect on the performance of the interferon gamma release assay, with a higher proportion of false negatives and uncertain results [6]. The patient underwent interferon gamma release assay during severe bone marrow suppression. At this time, the number of lymphocytes in the peripheral blood is extremely reduced. When there are not enough memory T lymphocytes, the secretion of interferon gamma also decreases, leading to a sharp decrease in the test results in a short period of time. So we contacted clinical doctors and advised the patient to undergo interferon gamma release assay after the patient recovered from bone marrow suppression. The result of retesting after the recovery of peripheral blood lymphocytes also confirm our hypothesis.

In summary, our case emphasizes that laboratory personnel should be proficient in the detection principles and influencing factors of interferon gamma release assay. When using Melphalan to treat patients with myeloma, attention should be paid to whether the patient has developed bone marrow suppression. When bone marrow suppression occurs, a negative interferon gamma release assay result cannot rule out potential tuberculosis infection. Laboratory staff should communicate with clinicians in a timely manner.

Table 1. Test results of interferon gamma release assay from the patient at different times.

Time	Interferon gamma assay result	Reference value
February 10, 2023	**802.4**	**0 - 20 pg/mL**
February 24, 2023	**3.94**	**0 - 20 pg/mL**
March 17, 2023	**168.5**	**0 - 20 pg/mL**

Table 2. Blood routine parameters of the patient at different times.

Time	WBC	Neutrophils	Lymphocyte
February 17th, 2023	**5.48**	**1.71**	**2.65**
February 18th, 2023	**3.74**	**1.95**	**1.05**
February 19th, 2023	**2.28**	**1.6**	**0.28**
February 21st, 2023	**3.3**	**3.21**	**0.03**
February 23rd, 2023	**1.5**	**1.5**	**0**
February 25th, 2023	**0.02**	**0.01**	**0.01**
March 14th, 2023	**4.28**	**0.56**	**2.85**
March 18th, 2023	**5.7**	**2.48**	**2.27**
Reference value	**3.5 - 9.5**	**1.8 - 6.3**	**1.1 - 3.2**
Unit	**10^9/L**	**10^9/L**	**10^9/L**

Sources of Support:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Interest:
All authors declare that they have no competing interests.

References:

1. Bentley SA, Ayscue LH, Watson JM, Ross DW. The clinical utility of discriminant functions for the differential diagnosis of microcytic anemias. Blood Cells 1989;15(3):575-82; discussion 583-4. (PMID: 2620101)

2. Zhu Y, Han S, Chen X, Wu S, Xiong B. Improving the diagnosis of myelodysplastic syndrome by red blood cell parameters. Clin Transl Oncol 2023 Oct;25(10):2983-90. (PMID: 37081223)

3. Arora RD, Dass J, Maydeo S, Arya V, Kotwal J, Bhargava M. Utility of mean sphered cell volume and mean reticulocyte volume for the diagnosis of hereditary spherocytosis. Hematology 2018 Aug;23(7):413-6. (PMID: 29338606)

4. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part I: platelets. Int J Lab Hematol 2007 Feb;29(1):4-20. (PMID: 17224004)

5. Roccaforte V, Sciarini F, Proserpio V, et al. Use of the reticulocyte channel warmed to 41°C of the XN-9000 analyzer in samples with the presence of cold agglutinins. Hematol Transfus Cell Ther 2021 Apr-Jun;43(2):147-55. (PMID: 32199923)

6. Lodi G, Resca D, Reverberi R. Fatal cold agglutinin-induced haemolytic anaemia: a case report. J Med Case Rep 2010 Aug 6;4:252. (PMID: 20691050)

7. Cunha BA. The clinical diagnosis of Mycoplasma pneumoniae: the diagnostic importance of highly elevated serum cold agglutinins. Eur J Clin Microbiol Infect Dis 2008 Oct;27(10):1017-9. (PMID: 18592280)

8. Berda-Haddad Y, Faure C, Boubaya M et al. Increased mean corpuscular haemoglobin concentration: artefact or pathological condition? Int J Lab Hematol 2017 Feb;39(1):32-41. (PMID: 27566136)