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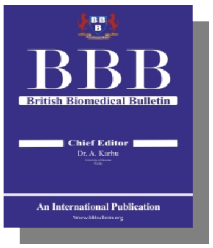

Original

Paraprotein-Induced Factitious Results on an Automated Haematology Analyzer

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A R T I C L E I N F O

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A B S T R A C T

Main findings: Paraproteins, due to their precipitating nature are known to artifactually influence various results ranging from haemoglobin estimation to immunological assays. We describe herein a case of multiple myeloma on treatment and follow up, which on routine analysis showed a spuriously high total leucocyte and platelet count. After decanting the plasma, these parameters receded to normal levels. The immunoglobulin assay confirmed the paraproteins.

Summary: Analysis of blood and its components by auto analyzers have simplified many a complex test. But few variables have to be borne in mind while scrutinizing the suspect messages flagged by the analyser. Some of these variables may be inexplicably linked to the patient's condition itself. And one of the most common variable is paraproteins seen in patients with multiple myeloma. This case aims to highlight some of the lesser known causes of pre-analytical errors concerning hemoglobin estimation by auto analyzers.

Potential implications: Paraproteins have been shown to interfere with a number of clinical laboratory tests due to their precipitating quality. Although seldom observed in routine diagnostic parlance, the possible clinical implications of such phenomena must never be overlooked.

Introduction

Paraproteins, also known as monoclonal immunoglobulins are proteinaceous secretions elaborated by neoplastic clonal plasma cells. Circulating paraproteins are intimately associated with multiple myeloma, also with

lymphoproliferative disorders and monoclonal gammopathy of undetermined significance (MGUS). The biophysical effect of monoclonal globulin is unpredictable since it depends on the immunological and chemical homogeneity along with its concentration. Being protein globules, they can precipitate at lower temperatures *in-vitro*. This property is also responsible to artifactually influence various automated analytical parameters such as blood cell counts, haemoglobin levels, immunological assays, bilirubin levels, electrolyte and other biochemical parameters.[1-9] This leads to misinterpretation of clinical laboratory results; subsequently leading to an incorrect diagnosis and inappropriate treatment. We herein present a case report of patient diagnosed with multiple myeloma (IgG type); showing interference in blood counts on a haematology analyser.

Case report

A 72 year-old male patient, diagnosed in 2010 as secretory (IgG) type of multiple myeloma based on bone marrow and biochemical findings. Chemotherapy was initiated with Melphalan. Four months after initiation of therapy, an appreciable decrease in immunoglobulin G (IgG) levels (1.85 g/dl) was observed. In May 2011, a follow-up showed a slight increase in IgG levels. The bone marrow biopsy revealed numerous aggregates of plasma cells and occasional plasma blasts; reported as myeloma not in remission. Hence patient was started on Bortezomib.

In November 2013, the patient presented with features of acute kidney injury with increase in urea (97mg/dl), creatinine (6.2mg/dl). The kidney biopsy showed features of acute tubulopathy with simplification of proximal convoluting tubules. The glomeruli were unaffected and did not show immunoglobulin deposits. Patient was treated with dialysis. On further follow up, the IgG levels remained same (4.126 g/dl). A complete blood analysis performed on the auto analyzer (Beckman coulter – LH 780^{TM}) showed the following: Total leucocyte count of 21.5 x 10^3/µl, with flagging of cellular interference (corrected counts of 3.9), red blood cell count of 2.31 x10^6/µl, haemoglobin 6.5 g/dl, haematocrit of 19.1%, platelet count of 254 x 10^3/µl. The corresponding peripheral smear showed an increase in rouleaux formation and left shift in neutrophil count with cytoplasmic vacuolations. However, the manual total leucocyte count (approximately 4.5x10^3 /µl) and platelet count (approximately 150 x10^3 /µl) performed on the smear did not tally with that of the machine. Thus, a common factor was affecting both these parameters, causing them to be elevated albeit spuriously. We suspected a possibility of a plasma factor. A spun hematocrit showed good agreement with the auto analyzer value. More importantly, the plasma layer appeared mildly turbid. The supernatant plasma was decanted and the blood cells were suspended in equal volume of normal saline. We ran the sample on another analyser (Sysmex-XP 100^{TM}). The comparison of the before and after counts are shown in table 1.

These counts showed a significant decrease in total WBC count, RBC count, haemoglobin and platelet count. These also corroborated with the manual peripheral smear report. Interestingly, the decanted plasma which was initially straw coloured; turned hazy and thick after 15 minutes at room temperature (figure 1). These findings, along with an elevated serum IgG level substantiated the paraproteins which had interfered with the blood counts in haematology analyser.

Discussion

The ever-increasing sample load in most laboratories have enhanced the application of automated analyzers for routine investigations. However, the potential of a variable affecting the final test result can never be ruled out. Analytical interference in the clinical laboratory is a well-known phenomenon and has different aetiologies. A quick scan through the available literature suggests a wide range of causes from exogenous sources such as drugs and chemical additives to endogenous substances such as haemoglobin, bilirubin and lipids.[10-12] Interferences caused by immunoglobulin are more difficult to test or anticipate, partly owing to their infrequent nature, but nonetheless are clinically important.

Among paraproteins, IgM and IgG are the most common immunoglobulins attributed for discrepancies in laboratory values. Most commonly seen in patients with multiple myeloma and Waldenstrom macroglobulinemia, the spurious haematology laboratory results are attributed to the formation of immunoglobulin precipitates i.e., formation of dense amorphous clusters, flake -like particles, needle like crystals, and pink globules, leading to abnormal increase in cell counts. The others may be due to cryoglobulins; precipitating at temperature < 37^{o}C, leading to abnormal cell counts mainly in haematology analysers that use reagents and perform counts at room temperature.[13]

Depending on their size, paraproteins can affect cell counts of all the three lineages. If cryoprecipitate or paraprotein form aggregates of larger sizes; they parallel the size of leucocytes; leading to spurious increase in WBC counts. Scatter-gram on a five-part differential count analyser in these situations show an abnormal scatter near or below lymphocytes.

Similar changes may be seen in platelet histograms, where giant platelets exceeding 20 femtolitres may be flagged, depending on the analyser used. Small particles can lead to spurious increase in platelet counts up to 8 times.[14,15]

Spurious high haemoglobin values can be explained; due to disturbance in light transmittance.[15] The estimation of haemoglobin using cyanmethemoglobin method is employs the colorimetric principle based on Beer's law, wherein the optical density of a coloured solution is directly proportional to the amount of substance present. Hence, any factor such as paraproteins, which increase the density of plasma can cause a falsely increased haemoglobin value. In this case the Hb reading was rendered as 6.5gm% as opposed to the actual 5.8gm% due to IgG molecules.

Sometimes abnormal low WBC counts, RBC counts, haemoglobin levels and platelet counts (pancytopenia) can be seen due to obstruction of flow of blood cells in the compartments of the analyser by large immunoglobulin molecules.[15]

Paraprotein associated factitious results can be minimised by the following methodologies[1]: 1) diluting the sample by reducing precipitates. 2) "De-proteinisation" of sample to reduce paraproteins. 3) Removing plasma and substituting it with equal amount of normal saline. 4) Setting up a manual flag to identify higher baseline absorbance. We removed the plasma from the sample, replaced it with an equal volume of diluent, and then analysed the sample in the normal manner. Thus, more appropriate parameters were rendered.

Conclusion

Paraproteins have been shown to interfere with a number of clinical laboratory tests due to their precipitating quality. We observed such interference

across many hematologic parameters in this case. Although seldom observed in routine diagnostic parlance, the possible clinical implications of such phenomena must never be overlooked.

Author's contribution

Dr. Indira Shastry K and Dr. Deepak Nayak contributed to conceptualisation of study, designed the study and helped in integration of data. Dr. Deepak Nayak also helped in analysis of data. Dr. Chean Manohar drafted the manuscript, helped in criticizing the intellectual content. Dr. Ravindra Prabhu and Dr. Joseph Thomas contributed in final approval of article.

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Table 1. Comparison of blood report before and after separation of plasma

Parameters	Before separation of plasma	After separation of plasma
Total WBC count(X 10^3/µl)	21.5	4.9
RBC count (X 10^6/µl)	2.31	2.16
Haemoglobin (g/dl)	6.5	5.8
Haematocrit (%)	18.0	19.1
Platelet (x 10^3/µl)	254	160

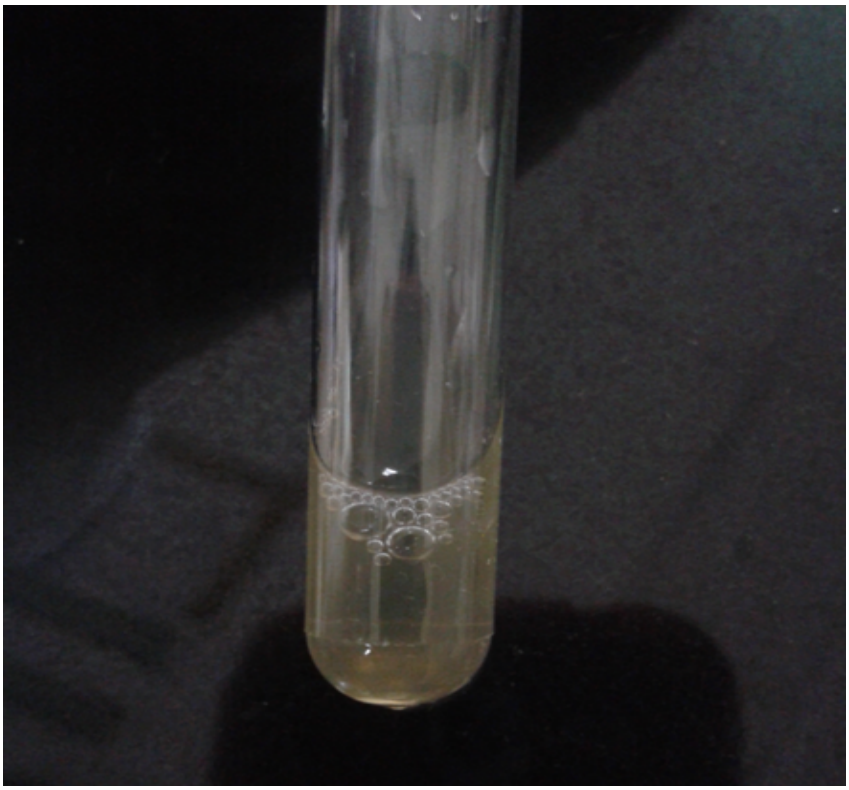

Figure 1. The decanted plasma from the blood sample, Note the turbid nature of the plasma