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Paraprotein-induced Discordance Between Direct and Total Bilirubin Levels in Patients with Myeloma: a Case Study Series

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Abstract— This study represents a preliminary investigation into the clinical implications of discordance between total and direct bilirubin levels in patients with multiple myeloma. Four cases are presented to highlight the variability in paraprotein concentrations and the discordance of total and direct bilirubin levels, emphasizing the importance of the timing of these measurements. The results of the bilirubin level discrepancy were observed either before the official diagnosis of multiple myeloma, or immediately after diagnosis, meaning before the initiation of chemotherapy for multiple myeloma**.**
Keywords— multiple myeloma, paraprotein interference, discordance, direct bilirubin, total bilirubin

I. INTRODUCTION

Overview of multiple myeloma

Myeloma or multiple myeloma is a neoplastic proliferation of a clone of plasma cells that produces excessive amounts of monoclonal protein (M-protein) and free light chains (FLCs). These monoclonal proteins, also known as paraproteins, can significantly affect various laboratory measurements due to their unique physical and chemical properties.

Impact of paraproteins on laboratory measurements

The presence of high levels of paraproteins in the bloodstream can lead to a range of diagnostic challenges, one of which is interference with biochemical assays.Paraprotein interference occurs when these proteins interact with the reagents or methods used in laboratory testing, skewing the results and potentially leading to misinterpretation. This can be particularly problematic in assays measuring bilirubin levels, an important marker for liver function and hemolysis.

Significance of bilirubin assays

Bilirubin, which exists in direct (conjugated) and indirect (unconjugated) forms, is usually measured to assess liver health and related disorders. However, in patients with multiple myeloma, the anomalous presence of paraproteins can result in discrepancies between total and direct bilirubin levels, complicating diagnostic evaluations and treatment plans.

Challenges and need for further study

Such interference poses a challenge for clinical laboratories and highlights the need for better understanding and methods to mitigate these effects. Studying the relationship between paraprotein levels and assay discrepancies may provide essential insights into refining diagnostic protocols for myeloma patients, ensuring accurate interpretation of laboratory results, and optimizing patient care.

II. METHODS

Patient selection criteria

This is a qualitative research that included purposive non-probability sampling. As not all physicians routinely assign forms of bilirubin for measurement, four cases of patients diagnosed with multiple myeloma wereselected through purposive sampling based on their bilirubin measurement results at MCSC n. a. A. S. Loginov from July to September. The primary criterion for inclusion was a documented instance of elevated direct bilirubin levels exceeding total bilirubin values.This unique selection was aimed at identifying cases where paraprotein interference might be present. The analysis initially included patients regardless of diagnosis, but further screening linked these discrepancies to individuals with potential or confirmed diagnoses of multiple myeloma.

Bilirubin measurement

Total and direct bilirubin levels were measured using Beckman Coulter Chemistry AU analyzers and Beckman Coulter reagents. The Beckman Coulter Direct Bilirubin Reagent utilizes a variation of the classical method developed by Van den Bergh and Mueller. In this method, direct (conjugated) bilirubin reacts directly with a diazonium salt of 3,5-dichloroaniline (DPD) in an acid medium to form azobilirubin. The color intensity of azobilirubin, measured bichromatically at 540/600 nm, is directly proportional to the concentration of direct bilirubin in the serum.

Presence of paraprotein

Serum protein electrophoresis (SPE) and immunotyping were conducted in the same laboratory department on the same day as bilirubin measurements, using the Capillarys-2 Flex Piercing Sebia analyzer. All cases exhibited paraprotein in SPE, aligning with the diagnosis or potential diagnosis of multiple myeloma. This indicates that paraprotein presence might play a role in interfering with bilirubin measurement.

Correlation uncertainly

The initial hypothesis suggested a dependence of bilirubin discordance on paraprotein concentration. However, no direct correlation was established, suggesting other influencing factors may be at play and warrant further exploration.

Review of medical histories

Medical records were reviewed to verify the patients' diagnoses and monitor the timeline of bilirubin discrepancies relative to the diagnosis of multiple myeloma and treatment initiation.

This case series aims to demonstrate the impact of paraproteins on direct bilirubin measurement in patients with myeloma and to illustrate the variability of paraprotein concentration across different cases. Upon reviewing the medical histories, it became apparent that discordant bilirubin levels were commonly found in patients who had not yet started treatment. This observation shifted the focus to a potential relationship between the timing of diagnosis, treatment initiation, and the appearance of bilirubin measurement discrepancies.

III. CASES

Case #1: Patient H.

Summary and initial observations

Patient H., a 74-year-old diagnosed with multiple myeloma on July 15, 2024, underwent laboratory tests three days later, on July 18, 2024. The results are presented in Table I and Fig. 1.

TABLE I

PATIENT H. LABORATORY TESTS RESULTS

Total protein, g/l	89,9
Total bilirubin, µmol/l	5,6
Direct bilirubin, µmol/l	8,1
M-protein immunotyping	IgG kappa
M-protein, %	28,4
M-protein, g/l	25,5

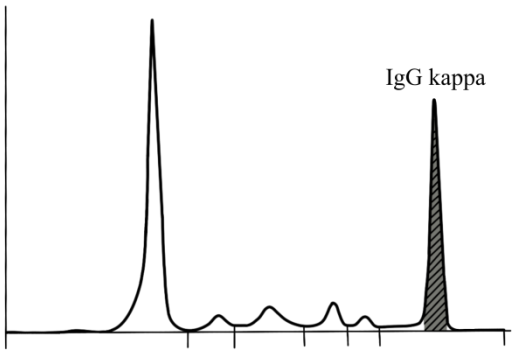

Fig. 1 Patient H. serum protein electrophoresis diagram

Discussion

This case highlights moderate bilirubin discordance. Although M-protein levels were significant, they were not the highest observed in the study.

Case #2: Patient K.

Summary and initial observations

Patient K., aged 70, was diagnosed with multiple myeloma on July 25, 2024, but exhibited signs of paraprotein-induced bilirubin interference before the diagnosis on July 11, 2024, and the results are presented in Table II and Fig. 2.

TABLE II

PATIENT K. LABORATORY TESTS RESULTS

Total protein, g/l	138,8
Total bilirubin, µmol/l	5,6
Direct bilirubin, µmol/l	6,1
M-protein immunotyping	IgG kappa
M-protein, %	57,8
M-protein, g/l	80,2
M-protein immunotyping	IgA kappa
M-protein, %	2,7
M-protein, g/l	3,3

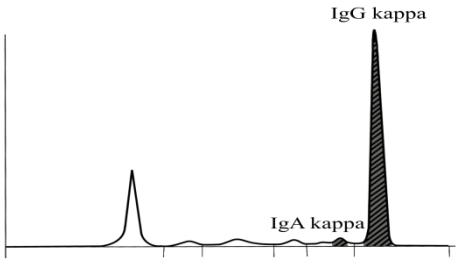

Fig. 2 Patient K. serum protein electrophoresis diagram

Discussion

Patient K.'s complex M-protein profile, with a dominant IgG kappa and a smaller proportion of IgA kappa, may have influenced the bilirubin assay outcomes. There is relatively slight bilirubin discordance observed despite high M-protein concentrations.Future studies could investigate how combinations of paraproteins influence assay accuracy compared to single-type paraproteins.

Case #3: Patient M.

Summary and initial observations

Patient M., a 68-year-old individual diagnosed with multiple myeloma on July 4, 2024, presented with notable pre-diagnosis bilirubin discrepancies on July 1, 2024.The results are presented in Table III and Fig. 3

TABLE III

PATIENT M. LABORATORY TESTS RESULTS

Total protein, g/l	130,5
Total bilirubin, µmol/l	2,9
Direct bilirubin, µmol/l	26,1
M-protein immunotyping	IgG kappa
M-protein, %	51,2
M-protein, g/l	66,8

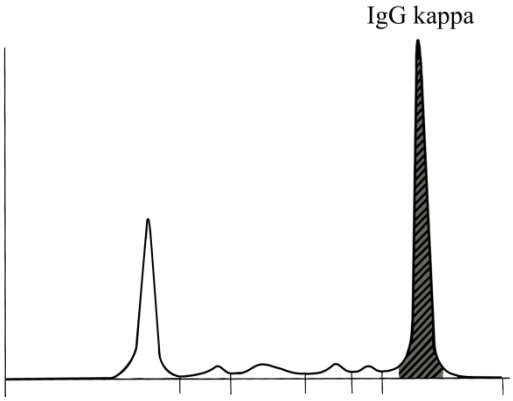

Fig. 3 Patient M. serum protein electrophoresis diagram

Discussion

Patient K. indeed has a higher paraprotein concentration than Patient M., yet the bilirubin discordance is less severe in Patient K. This confirms our finding that there is no direct correlation between paraprotein concentration and the level of bilirubin discrepancy.

Case #4, Patient U.

Summary and initial observations

Patient U., aged 57, was diagnosed with multiple myeloma on September 20, 2024, with pre-diagnosis tests conducted on September 15, 2024.The results are presented in Table IV and Fig. 4.

TABLE IV
PATIENT M. LABORATORY TESTS RESULTS

Total protein, g/l	92,3
Total bilirubin, µmol/l	9,8
Direct bilirubin, µmol/l	16,8
M-protein immunotyping	IgG lambda
M-protein, %	38,7
M-protein, g/l	35,7

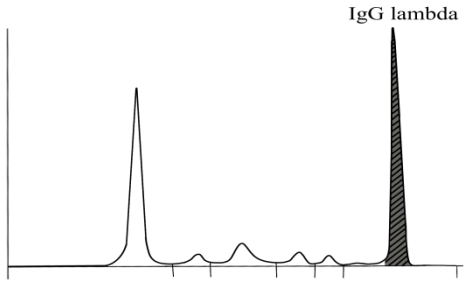

FIG. 4 PATIENT U. SERUM PROTEIN ELECTROPHORESIS DIAGRAM

Discussion

The different paraprotein type (IgG lambda) introduces a variable to consider when comparing this case to others with IgG kappa types. Investigating whether IgG lambda proteins have unique chemical interactions that alter bilirubin assay results differently than IgG kappa could provide further clarification.

Case comparisons

Case #2: Patient K. had the highest total paraprotein concentration at 80.2 g/l (IgG kappa)with an additional 3.3 g/l (IgA kappa).However, the bilirubin discrepancy was minor, with total bilirubin at 5.6 µmol/l and direct bilirubin at 6.1 µmol/l.

Case #3: Patient M., on the other hand, had a lower paraprotein concentration of 66.8 g/l (IgG kappa) but demonstrated a much larger bilirubin discrepancy, with a total bilirubin 2.9 µmol/l and direct bilirubin 26.1 µmol/l.

Case#1: Patient H. had a total paraprotein level of 25.5 g/l (IgG kappa) with moderate bilirubin discrepancy, showing total bilirubin at 5.6 µmol/l and direct bilirubin at 8.1 µmol/l.

Case #4: Patient U. had a total paraprotein concentration of 35.7 g/l (IgG lambda) and showed a more considerable bilirubin discrepancy with total bilirubin at 9.8 µmol/l and direct bilirubin at 16.8 µmol/l.

Comparisons insights

Case #1 vs. Case #4: Case #1: Patient H. and Case #4: Patient U. had lower paraprotein concentrations compared to Case #2: Patient K. and Case #3: Patient M., yet their bilirubin discrepancies were still present, though to varying degrees. Patient H. had a moderate bilirubin discrepancy, whereas Patient U. showed a more significant difference despite having a similar paraprotein level to Patient H.

Cases #2 and #3 vs. Cases #1 and #4: The comparison between these cases highlights that there is no straightforward relationship between paraprotein concentration and the level of bilirubin discrepancy.

This comparison suggests that factors other than the concentration of paraproteins contribute to the degree of bilirubin assay interference. Potential explanations might include:

- Paraprotein structure and composition: Different paraproteins may interact variably with bilirubin assays depending on their specific structural properties, such as isotype (e.g., IgG vs. IgA) or light chain type (kappa vs. lambda).
- Patient-specific biochemistry: Differences in individual patient biochemistry, such as liver function or other metabolic conditions, could influence how paraproteins interfere with bilirubin assays.
- Assay sensitivity and reactions: The way certain paraproteins react with assay reagents may not be solely dependent on concentration but also on how they bind or alter assay components.

IV. CONCLUSIONS

The interference caused by paraproteins in patients with multiple myeloma may result in discordant measurement of direct and total bilirubin, underscoring the necessity for further research to clarify the specific clinical implications and potential diagnostic complications of these observations. Each case demonstrated elevated direct bilirubin levels either before or soon after the diagnosis of multiple myeloma, but always before the start of treatment.This consistency indicates that untreated multiple myeloma may play a role in the interference observed in direct bilirubin measurements.Also understanding the influence of paraproteins on direct bilirubin measurement may be crucial for effective screening, allowing physicians to adapt their approach and make informed decisions regarding subsequent diagnostic evaluations and patient management strategies.

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