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Case Presentation

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Laboratory Physician's Role in Clinical Diagnosis: Report of a Case of Abnormal Increase in β2-Microglobulin in Blood

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Introduction

Laboratory medicine is indispensable for accurate diagnosis and treatment in clinical practice. Developments in basic medical research have greatly promoted progress in laboratory medicine, accompanied by the constant emergence of new investigative techniques, methods, devices, and indices. To ensure that these new investigative modalities are used in clinical practice both rationally and efficiently, laboratory personnel and clinicians must cooperate and communicate effectively. Also, feedback from clinicians in terms of clinical diagnosis and treatment helps promote laboratory medicine toward developing clinical diagnostic and therapeutic-oriented research. Thus, instead of a simple laboratory investigative process, clinical examination has gradually grown into the field of laboratory medicine from medical laboratory science, and is now a separate clinical department and discipline. Laboratory medicine combines investigation and clinical diagnosis based on biochemistry, immunology, microbiology, hematology, genetics, and molecular biology, among others. In this situation, the emerging role of the laboratory physician was born at the right time so as to facilitate the interplay between investigation and clinical practice. This paper builds its discussion around one clinical case, in which an abnormal increase in blood β2-Microglobulin (β2-MG) elicited inquiry from a laboratory physician, prompted and guided the clinical diagnosis, aiding in the definitive diagnosis. This paper demonstrates that laboratory physicians can play a bigger role in aiding the diagnostic process, guiding clinical diagnosis, and

making definitive diagnoses in modern medicine.

Case Presentation

The patient was a 61-year-old woman who was admitted to hospital with complaints of back pain that restricted her activities for over 1 month, and had recently worsened 10 days before presentation. She had pain in the chest, lower back, and upper back with no preceding trauma over one month earlier, mostly on the left side. The pain progressed to involve the left lower abdomen, and worsened when the patient sat up or was slightly relieved when she stayed in bed to rest. The pain was also relieved by taking Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). She had no fever or chills, no tightness in the chest, shortness of breath, nausea, or vomiting. About 10 days prior to presentation, the back pain recurred and had worsened. Also, it was no longer relieved by bedrest and NSAIDs. Thus, this patient came to our hospital for treatment. Since being successfully treated, she has not had any obvious fever, cough, stomachache, and shortness of breath. Stool test and urinalysis results have remained normal. The patient had no past medical history of hypertension, diabetes, kidney disease, or other cardiovascular or cerebrovascular disease. She had no drug allergy, had no associated injury, and did not smoke or consume alcohol. About 1 year earlier, she sustained a compression fracture of T12 and underwent Percutaneous Vertebroplasty (PVP) at another hospital. She recovered and was discharged from hospital. There was no family history of similar condition. On physical examination, her temperature was 36.8 °C, respiratory rate 20 breaths/min, pulse rate 70 beats/min, and blood pressure 120/80 mmHg. The patient had tremors at rest, involving the head and neck, and the limbs, but she was conscious, correctly responded to questions, and cooperated with the physical examination. There was no pallor or jaundice, no rash, or petechial hemorrhage and no open wound. There was also no significant peripheral lymphadenopathy. There was tenderness on the left chest wall, but no obvious abnormality was found on auscultation of the heart and the lung or on abdominal examination. There was no bilateral pitting pedal edema. X-ray from that previous hospital showed evidence of PVP in T12. We made an initial clinical diagnosis of osteoporosis with compression fracture of the thoracolumbar spine at T12 and evidence of PVP. The results of initial biochemical investigations are shown in **Table 1.**

Table 1: Biochemistry results.

Sample types	Test	Result	Reference
Blood	Potassium, mmol/L	3.34	3.5-5.3
	Sodium, mmol/L	141.1	137-147
	Chloride, mmol/L	108.7	99-110
	Total calcium, mmol/L	3.07	2.00-2.60
	Inorganic phosphorus, mmol/L	1.34	0.90-1.70
	Ca/P ratio	2.3	
	Ca*P, mg/dL	51.0	36-40
	Urea, mmol/L	15.9	2.8-8.2
	Creatinine, μmol/L	313	70-106
	Carbon dioxide, mmol/L	21.1	22-32

	Glucose, mmol/L	4.69	3.90-6.10
	Fructosamine, mmol/L	2.49	1.40-2.95
	Uric acid, μmol/L	514	155-357
	Cystatin C, mg/L	4.96	0.54-1.15
	β2-microglobulin, mg/L	**19.40**	**1.00-3.00**
	Alanine transaminase (ALT), U/L	8	7-40
	Aspartate transaminase (AST), U/L	18	13-35
	ALT/AST ratio	0.3	
	Glutamyl transpeptidase, U/L	43	7-45
	Total protein, g/L	67.1	65-85
	IgG, g/L	5.0	7.0-16.0
	IgM, g/L	0.14	0.80-4.00
	IgA, g/L	0.28	0.40-2.40
	C3, g/L	0.97	0.90-2.10
	C4, g/L	0.43	0.10-0.40
	Kappa, g/L	1.30	1.38-3.75
	Lambda, g/L	3.97	0.93-2.42
Urine	Kappa, g/L	0.10	0-7.1
	Lambda, g/L	2.86	0-21
	Kappa/ Lambda, g/L	0.03	1.17-2.93
	β2-microglobulin, mg/L	186.0	0.10-0.30
	Protein quantification, g/24h	6.76	0-0.15

QUESTIONS TO CONSIDER

1. The patient's serum β2-MG was abnormally elevated to almost the upper limit of normal.
2. The response curve must be reviewed before the examination report is released.

Laboratory Physician Analysis, Management, and Examination of Reports in General Quality Control

Quality assurance before analysis: The laboratory physician communicated with the nursing staff to ensure that no associated factors would influence the results in this patient. For example, samples were collection into appropriate dry bottles in accordance with relevant requirements, promptly delivered with no transport problems, and confirmed to show 3 clear fractions with no gross evidence of hemolysis, jaundice, or hyperlipidemia after centrifugation. No other influencing factors were found.

Quality assurance during analysis

The state of the biochemical analyzer was confirmed to be normal. Item examination results were normally distributed, and the reagents were free of contamination and efficacy loss. For the internal quality control of the investigation parameters, normal values and high values had controllable quality, and approximated the target values. The Coefficient of Variance (CV) in the current month was within the set CV value range (≤6%) and the external quality assessment reached required levels, with an average level free of considerable bias. Also, no

operational errors were found and the parameters were consistent with provisions as set forth in the standard operating procedure; the calibration was normal. For β2-MG assay, the sample was diluted using the double dilution method and the response curve and the final investigation results are shown in **Figure 1A.** The above figure shows that absorbance constantly increased after reagent 2 was added at the 10^{th} point in the investigation process. Subsequently from the 20^{th} to the 27^{th} point (target), the curve tends to be smooth, and the absorbance at the end decreased compared with that at the 25^{th} point. However, the response curve stays below the curve of the positive and negative wavelengths. The concentration of β2-MG antibodies in the commercial reagent are constant. However, the high β2-MG levels in the sample are in excess of the β2-MG antibodies in the reagent and this disproportionate antigen to antibodies ratio weakens the stability of the antigen-antibody complex, leading to the "hook effect". Thus, the actual β2-MG concentration of the patient is not reflected in this situation. Therefore, the patient's sample was diluted to one-eighth of the original and reexamined. The response curve of the diluted sample is shown in **Figure 1B.** The result of the dilution to one-eighth was 13.41mg/L, and the final concentration of β2-MG was 107.28 mg/L.

Quality assurance after analysis

The laboratory physician reported the investigation results to the attending physician and the head of department. After reviewing related medical records, the physician made the primary clinical diagnosis as follows: osteoporosis with compression fracture of the T12 thoracolumbar spine and evidence of PVP. The physician also reviewed the other blood chemistry results.

- Clinical biochemistry (Total calcium, Urea, Creatinine, Cystatin C, Carbon dioxide, Glucose in **Table 1).**
- Routine urinalysis: occult blood 3+, albumin 3+, and red blood cells 3+ on microscopy.
- Full blood count: White blood cells, total 5.37×10^{9} /L; differential count was within normal. Red blood cells (RBCs), 1.28×10^{12} /L; hemoglobin, 44 g/L. Platelets, 68×10^{9} /L. Blood film showed mature hypochromic RBCs and thrombocytopenia with a roughly normal morphology.

Based on a comprehensive analysis of the investigation results and patient information, the laboratory physician then made a preliminarily diagnosis of Multiple Myeloma (MM).

QUESTIONS TO CONSIDER

Why did total protein and A/G show no significant abnormality? Why was there an abnormal increase in globulin?

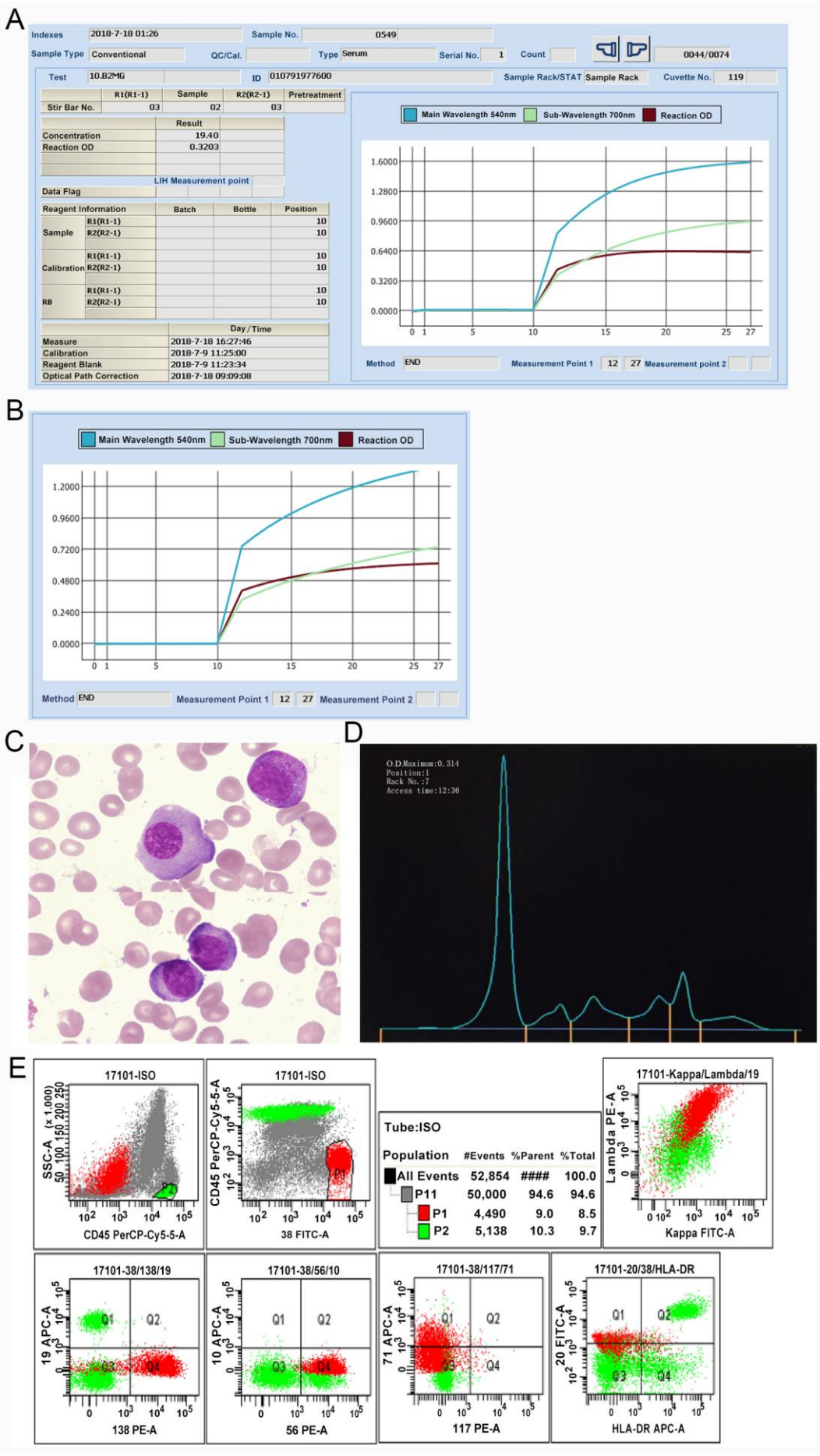
MM can be classified into eight types, based on the secretion of myeloma cells and the secretion of various monoclonal antibodies, as follows: IgG, IgA, IgD, IgM, IgE, light chain, biclonal, or polyclonal, and non-secretory types. Of these, MM of IgG, IgA, IgD, IgM, IgE, biclonal, or polyclonal types is accompanied by abnormal serum levels of myeloma (M) protein; the incidence of IgG-type MM is 50% and that of IgA-type is 15-20%. Light-chain monoclonal immunoglobulinopathy is either monoclonal κ chain or monoclonal λ chain, with absence of heavy chain; the incidence is 15%-20%. Non-secretory multiple myeloma has characteristic clinical manifestations, but there is no M protein in the serum; the incidence is about 1-2% **[1,2].** Thus, based on the above information, there was a high index of suspicion for Light-Chain Multiple Myeloma (LCMM).

Communication between the Laboratory Physician and the Attending Clinical Physician

The laboratory physician communicated with the attending clinical physician to propose the implementation of the following investigations to further confirm the diagnosis. This included bone marrow aspiration cytology, histochemical and flow cytometric analyses; bone marrow aspiration biopsy and histology; serum protein electrophoresis and serum immunofixation electrophoresis; seven blood immunological indices (IgG, IgM, IgA, C3, C4, light chain κ, and light chain λ); two urine light chain indices (light chain κ, and light chain λ; urine Bence-Jones Protein (BJP) qualitative test; urine β2-MG; erythrocyte sedimentation rate; and serum free light chain.

Verification of Inference and Diagnosis of the Laboratory Physician

Based on the symptoms, signs, imaging findings (including pathological fracture and osteolytic change), and all the outlined laboratory investigation results, the preliminary diagnosis of the laboratory physician was confirmed, and the patient was diagnosed as having LCMM (λ type). Bone marrow histology **(Figure 1C)** shows a plasma cell population of about 32.0% and 31.5% in the upper (young plasma) and lower (mature cells) panels, respectively. Here, the younger cells are larger in size, and round, oval, or irregular in shape and the cytoplasm is rich, dark gray, and foamy with a few eosinophilic granules. The nuclei are round or oval, eccentric, with coarse granular chromatin, and 1 or 2 visible nucleoli. **Figure 1D** shows the results of serum protein electrophoresis. Albumin, 0.508; α1-globulin, 0.075; α2-globulin, 0.134; β-globulin (with small abnormal peaks), 0.213; γ-globulin, 0.070. Flow cytometric immunophenotyping revealed an abnormal plasma cell fraction of 9.0% **(Figure 1E).**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Test 10.B2MG ID 010791977600 Sample Rack/STAT Sample Rack Cuvette No. 119
R1(R1-1) Sample R2(R2-1) Pretreatment
Stir Bar No. 03 02 03
Result
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Reaction OD 0.3203
LIH Measurement point
Data Flag
Reagent Information Batch Bottle Position
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R2(R2-1) 10
Calibration R1(R1-1) 10
R2(R2-1) 10
RB R1(R1-1) 10
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P1 4,490 9.0 8.5
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Lambda PE-A
Kappa FITC-A
17101-38/138/19
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17101-38/56/10
10 APC-A
56 PE-A
17101-38/117/71
71 APC-A
117 PE-A
17101-20/38/HLA-DR
20 FITC-A
HLA-DR APC-A
Q1 Q2 Q3 Q4

Figure 1: Examination results of patient. (A) Response curve of β2-MG assay. (B) Response curve of β2-MG assay after sample dilution to one-eighth of the original concentration. (C) Bone marrow examination (×100). (D) Serum protein electrophoresis test. (E) Flow cytometric immunophenotyping test.

QUESTIONS TO CONSIDER

β2-MG in urine was abnormally and significantly increased to 186.0 mg/L, and was higher than β2-MG in blood.

In serum, β2-MG is a microglobulin found on the surface of nucleated human cells, particularly lymphocytes and tumor cells [3]. It is a light chain of the human leukocyte antigen complex can be freely filtered by the glomeruli from plasma, and about 99% of these light chains are absorbed, decomposed, or destroyed by renal tubular epithelial cells [4]. Elevated serum β2-MG levels could indicate lymphoid neoplasms including lymphoma, myeloma, and lymphoid leukemia [5,6]. It could also be due to glomerular injury, decreased glomerular filtration rate, transplant rejection, HIV infection, infection, and other immune disorders [7]. In urine, β2-MG is a sensitive and specific diagnostic marker of proximal tubule injury. The ratio of urine protein to β2-MG in urine helps distinguish between glomerular or renal tubular lesions, and urinary tract infection [8,9]. An increase in serum β2-MG and concomitant normal or decrease in urinary β2-MG indicates glomerular dysfunction. In contrast, normal serum β2-MG with increased urinary β2-MG indicates renal tubular dysfunction. In addition, a simultaneous increase in β2-MG in both serum and urine indicates nucleated cell destruction in blood, and excessive β2-MG synthesis. Based on these results, the laboratory physician concluded that the patient had overflow proteinuria and increased β2-MG synthesis that surpassed the reabsorbing capacity of the renal tubules.

QUESTIONS TO CONSIDER

Why did light chain λ in urine increase significantly (2.86 g/L) whereas urine BJP was negative on qualitative urinalysis?

In this patient, urinary BJP was negative on urine BJP qualitative testing. From the final diagnosis of this patient, we can analyze this case as follows, First, before the analysis, the dilution of the urine sample could have led to a false negative result. Therefore, to determine whether this urine sample is diluted, the attending physician, the nurse, and the patient should all be educated about sample collection. During the analysis, the laboratory conducted this test using the thermal precipitation-dissolution method. This method takes advantage of the fact that BJP characteristically solidifies at 40 to 60°C and dissolves at 90 to 100°C. The methodology, however, has a low sensitivity, high false negative rate, and requires a large sample size. Given that the urinary BJP qualitative test is influenced by more factors, we recommend that Free Light Chains (FLCs) in urine or Immunofixation Electrophoresis (IFE) be performed instead.

Conclusion

The initial symptoms of a disease manifest in numerous forms. Therefore, clinicians determine the pathogenesis through history-taking, physical examination with the support of laboratory and other investigations. The

supporting role of laboratory investigations is vital for definitive diagnosis of a blood disorder. This case offers a typical example. This patient was admitted to hospital because of back pain that restricted her activities for over 1 month, which had worsened 10 days prior to presentation. She was preliminarily diagnosed as having osteoporosis. However, the laboratory physician identified a significant increase in serum β2-MG levels, and thus based on further laboratory examination it was discovered that the patient had hypercalcemia, renal dysfunction, and anemia. There was a high index of suspicion for MM and from the subtype and M protein analysis, the laboratory physician directly inferred that the class of MM was indeed LCMM. Finally, this diagnosis was confirmed. The laboratory physician notified the clinician of improvement in subsequent related investigations; then, based on clinical features and other related investigations, the laboratory physician made a definitive diagnosis of LCMM. Thus, the patient could receive appropriate treatment as early as possible.

In the diagnostic process, the laboratory physician should consider the investigation results for a particular case, and actively communicate with the attending physician clinician. Moreover, the laboratory physician should be familiar with the diagnostic criteria for various diseases, thus assisting the clinician in confirming the diagnosis of the patient. Therefore, as a bridge between laboratory investigations and clinical practice, laboratory physicians are not only high-caliber technicians, but are also physicians with comprehensive clinical diagnostic skills.

Author Contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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