REVIEWS

Application of serum protein electrophoresis in multiple myeloma

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Received: March 28, 2018 **Accepted:** May 11, 2018 **Online Published:** June 10, 2018
DOI: 10.14725/dcc.v5n2p23 **URL:** http://dx.doi.org/10.14725/dcc.v5n2p23

Abstract

Multiple myeloma (MM) is a disease characterized by abnormal proliferation of clonal malignant plasma cells (myeloma cells) in the bone marrow and associated with the increase of monoclonal immunoglobulins (i.e, M-proteins). As the incidence of MM has been increasing year by year, with diverse clinical manifestations, the early diagnosis of MM is of great significance to the subsequent treatment and prognosis. Serum protein electrophoresis (SPE) is the most simple and effective method to isolate protein with the advantages of clear electrophoregram and band, high resolution and good repeatability. It is currently the first choice for the diagnosis of MM. To study the application of SPE to MM can provide a certain reference and help for the preliminary immunophenotyping, diagnosis and efficacy observation of MM.

Key Words: Multiple myeloma, Serum protein electrophoresis, M-protein

Multiple myeloma (MM) is a fetal malignant B-cell tumor. Its onset is caused by the out-of-control and destructive growth of plasma cells that are mutated in the bone marrow.[1] The incidence accounts for about 10% of malignant diseases in the blood system.[2] Monoclonal proliferation of malignant plasma cells can produce a large number of monoclonal immunoglobulins (M-proteins). MM has a slow onset, with some common clinical symptoms such as multiple osteolytic lesions, anemia, infection and renal dysfunction. The clinical manifestations are diverse but not specific, so that misdiagnosis and missed diagnosis easily happen to patients in the early stage of MM.[3] Selecting an appropriate detection method is of great significance to the timely diagnosis of MM. Serum protein electrophoresis (SPE), used to isolate proteins,[4] can detect the abnormal production of proteins in the human body. It is a common method for the detection of serum proteins. Different diseases show different SPE electrophoregrams, and the characteristic performance of SPE in MM makes M-protein detection become one of rapid methods in the early diagnosis of MM.[5] Besides, M-protein detection is listed as one of main criteria in the current diagnosis of MM. Because SPE is widely used in clinical practice, with its own characteristics such as accurate results, easy operation and widely application, it has a certain significance to the early diagnosis, condition monitoring and treatment options of MM. However, SPE has a variety of limitations and differences in the diagnosis of different types of MM. In order to reduce the misdiagnoses and missed diagnoses of MM, the progress of SPE application to MM is reviewed in the following content.

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1 The pathogenesis and clinical characteristics of MM

MM is a common type of hematological malignancy. Studies have shown that some types of MM originate from the monoclonal gammopathy of undetermined significance (MGUS).[6] Myeloma cells in MM are derived from plasma cells in the bone marrow, and plasma cells originate from a type of B lymphocytes in leukocytes, which secrete antibodies to fight against infection normally. Therefore, MM can also be classified as a type of B cell lymphoma. Myeloma cells are derived from plasma cells, so MM is also a type of malignant plasma cell dyscrasias. The formation, differentiation and maturation of other blood cells in the blood are inhibited due to the abnormal proliferation of plasma cells. The number of blood cells with normal cellular morphology in the blood is reduced consequently. Therefore, immunoglobulins whose structures are different from the normal structures appear in the serum. Besides, plasma cells secrete antibodies abnormally, i.e., these antibodies are represented by globulins without immune function or some partial fragments, all of which are small proteins. When excessive substances of such kind appear in the serum, abnormal globulins are filtered from glomeruli, and then a large amount of immunoglobulin light chains can be observed in the urine. These abnormal antibodies that are distributed in the blood and urine are called M-proteins clinically. When M-proteins appear in the human body, the detection of M-proteins can be used as a clinical diagnosis basis.[7] However, not all types of MM produce M-proteins clinically, and the type of MM that does not produce M-proteins is called nonsecretory MM. Nonsecretory MM is rare to be seen clinically, with a lower prevalence. Most of patients with secretory MM can be classified into IgA, IgG, IgM, IgD or IgE as well as light-chain MM [mainly characterized by the abnormal proliferation of free monoclonal light chain (κ or λ)] patients. Current researches show that different types of MM may differ in the prevalence. The prevalence of IgG is highest among all types of MM, and IgA, IgM, light-chain and IgD MM are rare clinically. The incidence is related to the amount of each type of immunoglobulins it contains respectively.[8] The proportion of MM male and female patients is almost identical, and MM is common in people over the age of 40 years. The age over 65 years is a risk factor for the occurrence of this disease. MM is rare or seldom to be seen in young adults and children. The prevalence of MM has been on the rise in recent years. Commonly, the relevant manifestations of MM show increased level of serum calcium, renal dysfunction, anemia, bone damage, as well as target-organ damages such as amyloidosis.[9] Different types of MM also show different clinical symptoms. For instance, the secretion of IgG MM is characterized by inhibiting the production of normal immunoglobulins, and abnormal immunoglobulins have no antibody activity, so that the human body lacks antibodies with normal resistant and defensive function and then patients are susceptible to infection; Patients with IgA MM are prone to hypercalcemia, hypercholesterolemia and hyperviscosity syndrome since IgA easily induce the production of polymers; The age at onset of IgD MM is low, and patients with this type of MM are prone to anemia, hypercalcemia and extramedullary infiltration, with poor prognosis; The light-chain MM leads to an abnormal proportion of M-protein heavy chains, light-chain proteins can easily pass through the kidney due to their low molecular mass, and then deposited on renal tubular epithelial cells. Long-time deposition can result in degeneration of renal tubules, eventually leading to the impairment of renal function. The most common symptom related to light-chain MM is the impairment of renal function caused by λ light chains.[10] In addition, biochemical examinations conducted to MM patients show that the level of abnormal immunoglobulins is increased in the serum and half of Bence-Jones proteins in the urine are positive. Routine blood tests indicate that the level of hemoglobins is low or normal. The abnormal proliferation of plasmablasts or immature plasma cells can be observed in myelogram smears, with the proportion of abnormal plasma cells exceeding 10%, meanwhile, the proportion of plasma cells is increased with the development of the disease.[11] *X*-ray skeletal examinations show multiple bone defect areas or osteoporosis and pathological fractures. The current diagnostic criteria of MM are based on the guidelines of the World Health Organization, National Comprehensive Cancer Network and the International Myeloma Working Group, and combined with Chinese guidelines for the diagnosis and management of multiple myeloma, in order to make a definitive diagnosis.

2 The principle and the clinical application of SPE

2.1 SPE detection principle

SPE is a rapid and convenient detection method. It can isolate different types of proteins based on the differences in surface charge of serum proteins and analyze the changes in the protein level to determine a patient's physical condition. The patient's serum (samples collected within 24 hours are preferential) is spotted onto the agar plate.[12] Since each type of proteins in the serum has its own specific isoelectric point, the pH value of which is usually below 7.5, a buffer solution whose pH value is above the isoelectric point is required to ionize proteins in the serum into negatively-charged particles. Under the energizing condition, these particles move to the positive electrode. Different types of proteins have different quantities of electric charges and molecular masses. Besides, the speed of movement in the electric field also varies.[13] Five clear bands are shown on the normal electrophoregram. According to the distance at which proteins are moving, these bands are ALB band, α_1 band, α_2 band, β band and γ band respectively. The protein content and its shape represented in the elec-

 ISSN 2375-8449 E-ISSN 2375-8473

trophoregram are similar to "six" in gesture language.[14] At present, there are various types of SPE in clinical practice, and the commonly used electrophoresis techniques are cellulose acetate membrane electrophoresis, agarose gel electrophoresis, polyacrylamide gel electrophoresis, immune electrophoresis, etc., with roughly similar principles of operation.

2.2 The expression of different diseases on the SPE electrophoregram

SPE electrophoregram reflects the general state of proteins in the human body. Types and contents of proteins may vary from diseases and stages of a certain disease, with the electrophoregram also differing. The albumin band is more prone to change. Patients with liver diseases are characterized by the decreased level of albumins and the increased level of γ-globulins, which are more obvious gradually with the transformation of hepatitis to liver cirrhosis or liver cancer. This condition is associated with the inversion of A/G ratio (a type of hepatocyte synthesis disorder). In addition, abnormal and increased levels of albumins are common in negative acute phase responses, such as inflammation, sepsis, malnutrition, nephrotic syndrome, pregnancy, hemodilution etc.; and the decreased level of albumins is common in dehydration; the usage of certain drugs also affects the effect of albumins on the results, and the reexamination results often return to normal after drug withdrawal. α_1 globulins mainly include α_1 antitrypsins, α_1 lipoproteins, α_1 acid glycoproteins etc., as well as a small amount of alpha fetoproteins. Generally, the increase of α_1 band is common in high alpha-fetoprotein conditions, such as liver tumors, germ cell tumors, pregnancy, inflammatory conditions. The decrease of α_1 band is common in α_1-antitrypsin deficiency and liver diseases, and this type of decrease is more obvious in severely impaired liver function and associated with the severity of liver disease. Therefore, the content of α_1 proteins has reference significance to the development and prognosis of liver diseases. α_2 globulins are mainly consisted of haptoglobins and α_2-macroglobulins as well as ceruloplasmins. The increase of such proteins can be seen in renal diseases, inflammatory reactions and severe diabetes; the content of these proteins shows no obvious changes in the early stage of hepatitis, while it is decreased gradually in the late stage and decompensated liver cirrhosis, and it is also common in hemolysis, malnutrition and hepatolenticular degeneration. β band, divided into β_1 and β_2, mainly contains transferrins and lipoprotein fibrinogens. It is associated with diabetes, hyperlipoidemia, fatty liver, nephrotic syndrome and malignant tumors. Usually, the increase of these proteins is accompanied by the increase of α_2 globulins; the decrease of β band can be observed in the late stage of liver diseases. γ globulins mainly include IgG, IgA, IgM, C-reactive proteins and other substances. The increase of such proteins can be seen in liver diseases. In addition to the decrease of albumins in liver cirrhosis, levels of IgG, IgA and IgM increase simultaneously, with the integration of β and γ bands and the appearance of β-γ bridge. Moreover, different degrees of the increase of γ globulins can also appear in acute myocardial infarction and autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, etc.[15]

2.3 The performance of SPE electrophoregram in MM

In patients with MM, plasma cells produce a single type of immunoglobulins due to malignant monoclonal proliferation. Since these immunoglobulins have identical quantity of electric charge and molecular mass, they can move to the same position on the agar-gel plate. A compact band can be observed in the electrophoregram by visual inspection, and high protein peaks with narrow bases are represented with the help of a scanner.[16] M-proteins are mainly located in γ band, other M-proteins are located in β_2 band, and the rest (small amount) is in other bands.[17] Some literatures on MM immunophenotyping show that[18, 19] different types of immunoglobulins differ in the quantity of electric charges, molecular mass and location. For example, IgA proteins are mainly in proximity to the distal end of the negative electrode, β band and β-γ band. Since the electrophoretic velocity of IgG proteins is slower than IgA proteins, IgG proteins are mostly located in γ band and β-γ band. At present, agarose gel electrophoresis is widely used, and it is better in clarity, high resolution, repeatability than cellulose acetate membrane electrophoresis. It can be used to observe various bands of proteins, and then scanned and detected by use of the matched scanner to clearly observe the full view of protein distribution, and calculate the percentage of each type of proteins and the percentage of M-proteins. To this end, SPE can roughly evaluate the changes in immunoglobulin type and the characteristic performance for monoclonality in different diseases, especially in the diagnosis of MM patients, SPE has become a basic detection method to diagnose MM by its characteristic performance.[20]

3 The application of SPE to MM

3.1 The application and significance of SPE to the diagnosis of MM

SPE is a preferred method for isolating proteins. It is easy to operate. The complete operation procedures include spotting, electrophoresis, staining, decolorization and scanning. It can be finished within 90 minutes.[21] It is necessary to make an analysis of different protein electrophoregrams after obtaining the comprehensive protein electrophoregrams. MM shows diverse clinical manifestations, with no typical clinical symptoms. Common MM examination and di-

agnostic methods include blood routine examination, biochemical examination, imageological examination, cytological examination of bone marrow, chromosome in situ hybridization, fluorescence in situ hybridization and other biological examinations. Routine biochemical and blood tests are of high sensitivity, but lack specificity. Only half of Bence-Jones proteins in the urine are positive. From the perspective of imageology, the ostealgia symptom is not obvious in patients under the mild state of disease or in the early stage of disease, and bone destruction shows no obvious changes in the early stage as well. It is much easier to detect lesions by use of CT and MRI than *X*-ray examination. These imageological methods have different degrees of sensitivity, and they are sorted in descending order as follows: PET-CT, MRI, CT and *X*-ray. Although cytological examination of bone marrow is with a high accuracy, it is an invasive examination and it can not be guaranteed to extract myeloma cells just by one-time puncture. Sometimes, it is needed to repeat this procedure for many times, so that this type of examination brings about a lot of inconvenience to the clinical work, which is also the cause of low rate of clinical diagnosis and high rates of misdiagnoses/missed diagnoses.[22] The misdiagnosis rate can reach up to 54% to 69%, about 2/3 of patients with MM are not made a definite diagnosis until the middle or late stage, to the extent that they miss the best timing of treatment. At present, the research application in the biological area (immunity and genetics included) has been more and more complete. However, the experimental process is so complex, time-consuming and limited that it is difficult for general hospitals to carry out relevant experiments. Nevertheless, SPE is widely used, and it is crucial to assist the early diagnosis of MM as it is simple, rapid and accurate.

3.2 Limitations of SPE to MM

With the optimization of diagnostic techniques, SPE has been more and more widely used in the diagnosis of benign or malignant diseases. However, in the diagnosis of certain diseases, the increased or decreased degree of normal proteins, or the content of malignant protein secretion and its distribution in the band, greatly affects the electrophoregram results, easily resulting in misdiagnoses and missed diagnoses. For example, in the case of MM diagnosis, when the M-protein peak is distributed in β-γ, β or α_2 band, M-proteins are not easily observed on the agar plate, and the decrease in the M-protein content is not easy to be observed and diagnosed. In addition, the level of serum globulins is not increased in patients with light-chain MM, nonsecretory MM or rare MM. It is required to determine the immunophenotype of MM by detecting levels of IgG, IgA and IgM serum proteins.[23]

Although the electrophoretograms of proteins in MM patients are characteristically different from those in other diseases, these electrophoretograms are poor in characteristic performance with a decreased diagnostic rate due to disease type and development in the course of clinical research. For example, M-protein peaks are not shown in the SPE electrophoretograms from patients with simple BJP (light-chain disease) type MM and nonsecretory MM, making it difficult for the clinical diagnosis. This type of nonsecretory MM[24] accounts for 1% to 5%. Because not a single type of abnormal immunoglobulins is produced in the serum, the electrophoresis results are negative. Moreover, patients may show anemia, ostealgia, infection and other common symptoms, but these clinical symptoms are not typical until they are confirmed by use of bone marrow aspiration in the late stage, so that it is prone to missed diagnosis even though MM of this type is rare; Light-chain MM is also prone to missed diagnosis as the content of M-proteins is low in these patients and M-protein peaks are not obviously shown in part of protein electrophoretograms. On the other hand, the presence of M-protein in protein electrophoresis can not be used in the diagnosis of MM. For instance, some diseases can lead to the increase in macroglobulins, transferrins, hemoglobins etc., so that the protein electrophoresis shows a high sharp M-protein peak with a narrow base while biochemical tests return such results that serum immunoglobulins are decreased quantitatively or remain to be normal. Therefore, protein quantification is used to differentiate from other diseases clinically. As to M-proteins, it is a single type of immunoglobulins secreted by B lymphocytes or plasma cells. Clinically, monoclonal gammopathies cover a variety of diseases, among which, malignant plasma cell dyscrasias that are commonly seen contain MM, Waldenstroms macroglobulinemia, malignant lymphoma, B-cell lymphoblastic leukemia etc.; Secondary monoclonal gammopathies include MGUS, cryoglobulinemia etc.; benign monoclonal gammopathies contain connective tissue disease, kidney disease, diabetes, cardiovascular disease etc.[25] In summary, the clinical diagnosis of MM needs to be combined with plenty of methods. SPE, urine Bence-Jones proteins screening test, immunofixation electrophoresis, serum free light chain detection are all common methods used in the diagnosis of MM. Urine Bence-Jones proteins screening test is often used for screening, with a lower sensitivity; The detection sensitivity of SPE is 500-2,000 mg/L;[26] The detection sensitivity of immunofixation electrophoresis is 100-150 mg/L, and it can be used to simultaneously observe different types of M-proteins (IgG, IgA, IgM, IgD and IgE), meanwhile, this method is able to detect an even lower content of monoclonal components and used for the early diagnosis of diseases.[27] Therefore, immunofixation electrophoresis is more perfect on the basis of protein electrophoresis, so that it can be used to define the type of M-proteins; The detection sensitivity of serum free light chain is high, and the detection of serum free light chain method is highly sensitive, and has a high clinical value in the diagnosis and prognosis evaluation of MM in

the preliminary diagnosis. Patients with obvious abnormalities in serum free light chains may have a higher tumor burden and a stronger invasiveness, which often indicates that patients have a poor prognosis.[28]

3.3 The application of SPE to rare type of MM

In the rare type of MM, the light-chain MM accounts for about 10.00%[29] and the IgD MM accounts for about 2%.[30] The rare type of MM has a variety of patterns. MM patients are prone to misdiagnosis as they have no specific clinical symptoms. Due to the low prevalence and unobvious clinical symptoms, patients with rare type of MM neglect the condition in the early stage, leading to missed diagnosis, misdiagnosis and delayed treatment, which affect the prognosis consequently. However, SPE can only figure out the presence of M proteins without the typing of abnormal, and it is of a greatly decreased sensitivity especially in the diagnosis of complex rare phenotype,[31] the content of γ globulins is low in the light-chain MM, and no M-proteins appear in the protein electrophoresis usually. M-proteins are mainly present in severe patients with high tumor burden; IgD MM is rare with a low incidence, and there are few relevant research literatures about it. In the quantification of IgD, the content of IgD is often higher than the normal value, and IgD quantitative detection is of great value in the efficacy evaluation and prognosis to patients with IgD MM.[32] However, SPE often does not show M-proteins; Biclonal MM accounts for 1% to 5% among all types of MM, and the most common combination is IgG-IgA (53%), followed by IgM-IgG (24%), with a more complex protein electrophoresis.[33] In summary, the application of protein electrophoresis to rare MM patients to make a diagnosis should be combined with immunofixation electrophoresis and immunoglobulin quantification to increase diagnostic accuracy, which is widely used in the diagnosis of MM typing currently.[34] In addition, as to the presence of free light-chain M-proteins, it is probably the result that light chains are bound to IgD or IgE type M-proteins. Therefore, immunofixation electrophoresis can be used as a screening method for the presence of M-proteins in rare MM patients.[35]

4 Conclusions

MM is a progressive disease caused by the malignant proliferation of plasma cells, and M-protein is specific in MM patients. Five bands are shown in SPE electrophoregram. A compact band can be seen in the electrophoregrams from MM patients. After being scanned, the electrophoregram will show a single peak of M-protein with a narrow base. Those bands are distributed differently in the electrophoregrams from different types of MM. The onset of MM is concealed, with clinical manifestations diversified. It is prone to missed diagnosis or misdiagnosis due to the complication of first-visit department. Auxiliary examination methods for the diagnosis of MM are diversified. In addition to biochemical tests, routine blood tests, myelogram smears, imaging, chromosomal and genetic tests, SPE method has become one of the important methods for the auxiliary diagnosis of MM.[36] Therefore, when clinical symptoms that are common but not typical appear clinically, SPE should be carried out in time. If M-protein band is found, it is recommended to combine with urinary protein tests and continue to conduct immunofixation electrophoresis, bone marrow aspiration, *X*-ray examination, MRI and other tests. Even if the results are proven to be negative, it is also required to make a regular follow-up and tracing observation, which plays an important role in the early diagnosis, the decrease of missed diagnoses and misdiagnoses and prolonging survival of patients.[37] In summary, SPE is not an alternative method to MM for the diagnosis of M-proteins, and it should be used flexibly to make it an important detection method for the early diagnosis and screening of MM.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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