BIOCHEMICAL CHANGES IN THE MULTIPLE MYELOMA

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Abstract: The research of the myeloma proteins peculiar for their homogeneity lead to a thoroughgoing study of the normal immunoglobulins, of their structure and metabolism. Immunoglobulins migrate electrophoretically with the fractions : alpha, beta or better gamma. The heavy and light chains are separately synthesized in plasma cells, and then binded , their synthesis being equilibrated, but, in diseases such as multiple myeloma,it may exist a great excess of light chains, that emerge in the urine as Bence –Jones proteins, next to which it can be noticed the presence in the blood serum in high quantity of one of the classes of G, A, D, E immunoglobulins. The variation of the serum proteins have been studied through the electrophoresis method on paper or in agar, noticing the albumins diminution, the occuring of a high and narrow wave peculiar for the disease in the sorroundings of the gamma globulins. The electrophoresis on agar gel from the serum snd urine, points out a paraprotein, which looks like a homogenous band, well-controlled, highly coloured, situated in the gamma area, scarcely in the beta or alpha areas. The peculiar biochemical indeces have been surveyed.

INTRODUCTION

The term of myeloma was given by Rustizky in 1873; Other researchers have named the disease in various ways: Zahn-pseudo leukemia, Naegeli – generalized injury of the marrow, Apitz –suggests the term of plasmocytoma. Taking into account the pathological proliferation of these elements, the term has been adopted by the Anglosaxon authors. Tohether with the study of the variation of plasma proteins, within this disease have occured the terms of gamma, alpha and beta plasmocytoma. The objectives or the aim of the research have consisted of : The study of the evolution, the manner and the duration of manifestation of the multiple myeloma; The observation of the morphological, physiological and numerical modifications of the plasmocytes during the disease; Establishing the incidence of the disease occurance, based on age and gender; The surveyance of some biochemical indexes’ variations at the patients suffering of plasmocytoma.

MATERIALS AND METHODS

The examination of the bone marrow (myelogram): Bone marrow samples can be obtain by medullary puncture. The selected locations for medullary puncture on adults are: the breast bone, the iliac crest and the cannon bone. Within the multiple myeloma (wityh plasmoblasts and plasmocytes) and within chronic infections, there is an exetended number of plasmocytes with a normal semblance. A complete and correct myelogram will cover the total number of the nucleated elements: normal 50 000- 100 000/ mm^3, the myelocyte and eritrocyte series ratio: normal 3/1 ; the ratio between myelocyte series and medullary lymphocytes : normal 10/1; the ratio between myelocyte series and reticulo-histiocyte elements : normal 10/0,5-1. The date thus achieved bring new definitions which can not always be offered by the exam of the peripheric blood.

The puncture of the hematopoietic bone marrow: The bone marrow puncture consists of penetrating the bone, getting into the medullary channel and processing through aspiration of the medullary juice. Medullary puncture offers conclusive information for the diagnosis of the following disorders: meganoblastic anemia, hemolytic anemia, osteomyelofibrosis, plasmocytoma, medullary metastasis.

Bone marrow smear execution : The medullary extracted juice is leaked over an inclined glass plate to separate the tissue fragments. The extracted material is deposited on a watch glass. With the fang of a blade pick up the fragments and depose them one by one on 4-5 blades. Then with a polished blade pick up a fragment with which a smear is executed, according to the blood smear technique. It is important to work fast , because the medullary juice coagulates fastly. The coloration is made after the usual technique, that of May-Grunwald-Giemsa’s. Another helpful procedure to examine the medullary fragments(especially in the hypoplasia’s case) consist of the isolation of some medullary fragments which, pressed between two blades, are laid out in a thicker layer.Thus a higher cell density is acquired and a microscopical image close to a bioptic one is created.

Immunoglobulins and their structure: The immunoglobulins are polycatenar proteins resulted from the combination of a two polypeptide chains with a various molecular weights and sequence of amino acids:the heavy chain (with a heavy medullary weight) and the light chain(with a light nedullary weight). The 5 classes of immunoglobulins differ from each other by a different sequence of the „H” chain, symbolized with Greek letters equivalent to those that symbolize the class

:the „Y" chain for the immunoglobulin G, the„μ" chain for immunoglobulin M, the„α" chain for the immunoglobulin A, the „δ" chain for the immunoglobulin D, the „ε" chain for the immunoglobulin E. There two different types of chains: „L" Lambda type (λ) and Kappa type (k): within a immunoglobulin molecule there can't exist in the same time the both types of chains. The two light types of chains and the heavy chains differ from each other through the primary structure of the amino acids.

Immunoglobulins subunits: Both the heavy and the light chains are separetely synthesized into plasmocytes and after that are bounded. Their synthesis is equilibrated to normal. Within diseases such as myeloma, there can be a huge excess of light chains which emerge in the urine as Bence-Jones proteins. They are formed just of light chains (Bence-Jones proteins) or from fragments of heavy chains (heavy chains disease). Bence-Jones proteins are found in the meyloma diseaseds' urine or pathologic macroglobulinemia, sometimes in high quantities, under the shape of dimerised „L" chains, bounded through disulphidic links.
It has been pointed out a great number of cases of myeloma in which the presence of Bence-Jones proteins is not associated with a higher level of immunoglobulins in blood.
The main characteristic of the myeloma proteins, and also of the Bence-Jones proteins, is that they are omogenous being synthesized by the plasmatic cells whic come from the malignancy of a single clone. Because of this, the myeloma proteins have been called *monoclonal* immunoglobulins, and the diseases they determine, *monoclonal gammopathies*.
There have been identified two types of Bence-Jones proteins, Kappa and Lambda, with a chemical structure, properly chemical and antigenic distinctive. In the urine or blood of the patients suffering of monoclonal gammopathy there can be found Bence-Jones proteins, either of Kappa type or lambda one, unlike the normal cases where the „L" chains from urine (in small quantities) belong to both types.
Monoclonal gammopathies – are diseases characterized by the presence in the serum or urine of monoclonal immunoglobulin, also called *paraproteins* or *component M*(monoclonal). This monoclonal component is produced by a single clone of lymphocyte cells, having a limited electophoretic mobility, occuring at the electrophoresis of the serum proteins like a narrow peak band. Physiologically, the plasmocytes produce heavy chains and light chains, much more, unlike the heavy chains. In the case of the myeloma with immunoglobulins G, 75% of patients present an excess of light chains which excrete into the urine, causing the appearance of the Bence-Jones-like proteinuria. In some other cases there is no production of heavy chains, being present just the light chains excessively (light chains disease). There are rare cases, anyway, where the myeloma cells don't excrete niether heavy nor light chains, due to either the synthesis incapacity or to the secretory block, these cells being non-secretory it could be about a non-secretory myeloma.

Myeloma proteins with an antibody activity: myeloma proteins are the only omogenous immunoglubulins that occur in the human blood, as a result of some neoplastic processes settled in the cells that form antibodies or in the mice, as a result of the induction of some plasmocytes with the help of mineral oils. Neoplasic processes reach to the active cell which produce antibodies for every micromolecular substances (K_3 vitamin) or macromolecular (bacteria's polysaccharides intestines).
Hypercalcemia- is frequently met at the multiple myeloma (20-35% of cases) reaching to some values of 12-16mg/100 ml serum, growth that is related to the processes of bone destruction, hyperparathyroidism secondary to renal insufficiency. The hypercalcemia from the multiple myeloma is accompanied by an increase of the blood phosphorus, and the serum alkaline phosphatases have normal limits.
Urine's exam – it is pointed out through the presence of the Bence-Jones protein in 40-50% of cases, even to 60%, when it's marked out electrophoretically.
Bence-Jones protein could have been isolated electophoretically and cromatographically. It migrates with the speed of the immunoglobulins„β" or „γ", or mediate.
Proteinemia - in the blood serum and plasma, the whole quantity of proteins is about 23,3g%(an average 9g%), increase due to the globulins, albumins generally being normal or low. These proteins being in excess, except the hyperviscosity they induce, have the property to accede to the surface of the circulating blood cells, thus explaining some haematological modifications and clinical symptoms; thus, due to the adhesion on the surface of the haematies, images of rolls of money appear on the blood smear. The amass of the Bence-Jones proteins in the renal tubes explains the manifestations of renal insufficiency.
Total proteins determination: To determine the total proteins, the uric acid, the urea, and the calcaemia it has been used the "Vitros Chemistri"device. The objective of the utilization: the TP blades measure the total proteins from serum and plasma. The display and the explanation of the test: serum proteins carry the medicines and metabolites, keeping the plasma's osmotic pressure. Most of the serum proteins are used in the liver, excepting globulins β. One of the most important serum proteins produced in the liver is the albumin.

Method's principle: the TP blade from the Vitros is dried , the analytic element is being covered with a polyester support. The analysis method is based on a reaction that produces a violet colour when the protein reacts with the copper

ion (Cu^{2+}) in an alkaline surrounding. The complex formed quantity of coloration is in proportion with the total quantity of proteins from the blood sample and it is measured through spectophotometric refraction. O drop from a patient blood is deposed on a blade and homogenous spread, setting the blade under the beneath blades. When the liquid penetrates the reactive layer, it responds to the protein. Test's type: colorimetric. Wave length : 540nm. Analysis' working time and temperature: about5 minutes on 37^0C. Reactives (blade ingredients) : copper suphate, tartaric acid, lithium hydrate. Another ingredients consists of polymeric drops, bands and surfactant. Blade's name: the external board of the cartridge contains a label with the test name, the name of the blade's lot, the expiry date and the storage temperature. Blade's preparation: take the blade; it has to have the room's temperature 18-28^{0}C before it is unfold and put it in the blade support. The cartridge is left to get warm at least 60 minutes after it had been removed from the freezer, and 30 minutes after it had been removed from the fridge. Flick the external shell of and it is immediately put in the blade support. The cartridge should be left at the room's temperature with 24 h before it's used. Normal values: 6.3-8.2g/dl.

Uric acid determination: The aim of the use: URIC Vitros blades measure the content of uric acid in the serum, plasma and urine. Display and explanation of the test: the uric acid is the final product of the purine metabolism.

Calcium determination : The aim of the use: the „calcium” Vitros blades measure the calcium concentration from the serum, plasma and urine. The display and explanation of the test: calciun is the bone major mineral component; 99% of the amount of calcium is to be found in bones.”Calcium” ions have an important role in the transmission of the nervous impulses and in the normal syncopation of the muscles.

Urea determination: The aim of the use: Urea Vitros bladesmeasure the urea concentration from the serum, plasma, and urine. The display and explanation of the test: the highest excretion of nitrogen in the form of the urea, which is synthesized in the liver, releasing it in the kidney.

RESULTS AND DISCUSSIONS

The sudy of the myelogram is the current method of analysis of the hematopoiesis. No matter what kind of myelogram is being done, this is a truthful account of the myelopoiesis, only if it expresses all its functional relations, synthesized in indexes and specific parameters.

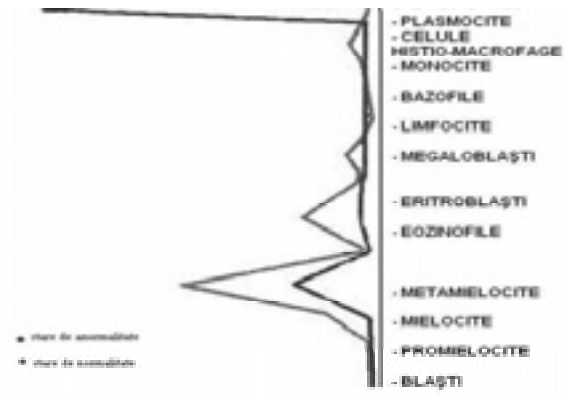


Figure 1: Myelograms in graphical representations (after Gowaerts) (M.TITEICA, 1984)

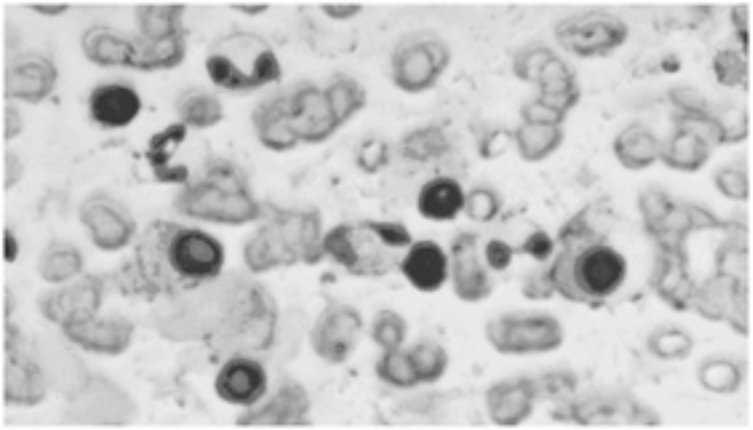


Figure2: Smear bone marrow (multiple myeloma) – original (20x40)

Peculiar to the smear bone marrow is the above figure, where it can be noticed the presence of the haematies in rolls and atypical forms of myeloma plasmocytes (plasmocytes in form of barbell)

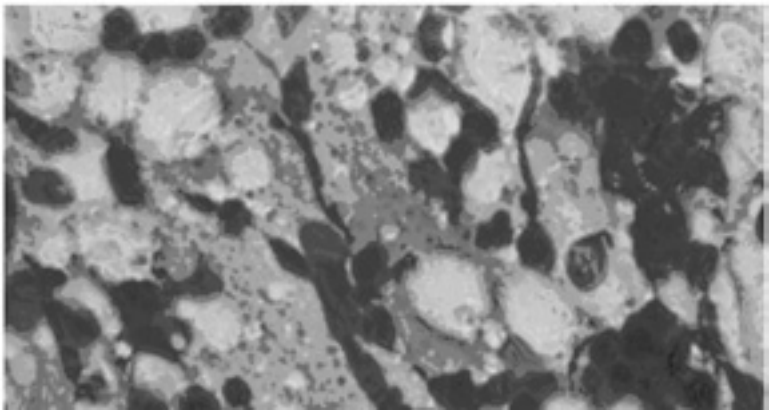


Figure3: Smear bone marrow (multiple myeloma) – original (20x40)

Within the multiple myeloma, the smear points out a period of massive invasion of the bone marrow with myeloma plasmocytes presented among the fragments of marrow, the presence of haematies being less noticeable, with a tendency to display in rolls.

Immunoglobulin are serum globulins which migrates electrophoretically with the „α", „β" fractions, and especially with „γ"fraction. The electrophoretic analysis of the serum has permitted the separation and measurement of the gammoglobulins, later called immunoglobulins(ig). Through the immunochemical analysis , the proteins heterogeneity has been proved, today being known 5 classes of immunoglobulins which, after a descending order of the normal proportions from the human serum are: Ig G, Ig A, Ig M, Ig D, and Id E.

Besides the Bence-Jones proteins, at the patients suffering of myeloma, sometimes it can be noticed the presence in the blood serum in some unusual high quantities of an one of the classes of immunoglobulins A, immunoglobulins G, immunoglobulins D or E; in the macroglobulinemia Waldestrom is presented immunoglobulin M.

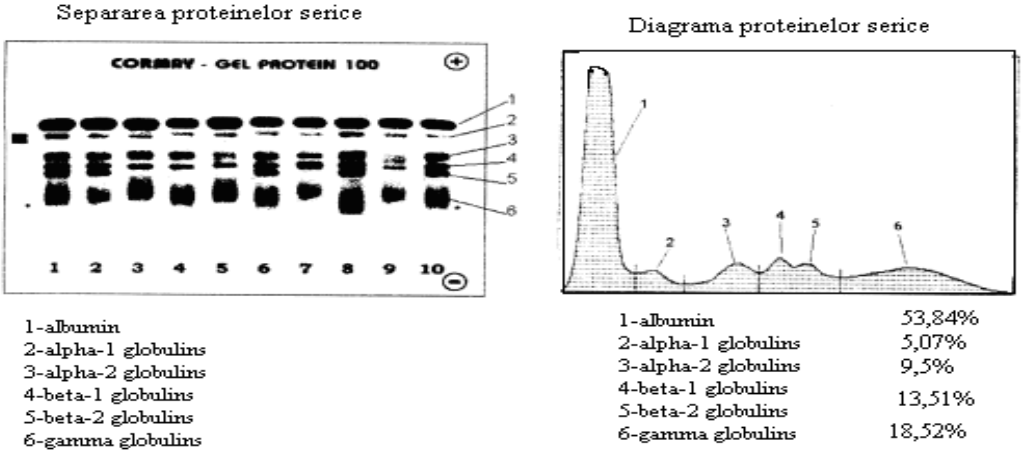


Figure 4: The separation and the diagram of the serum proteins(original)

The plasma globulins represent a heterogenous class of proteins, through electrophresis being separated the se α_1, α_2, β_1, β_2, γ globulins. Through electrophoresis, it could be obtain the separation of the γ globulins into two fractions γ_1, γ_2 , the later one presenting a faster migration to the electric field. From a chemical point of view, globulins γ don't contain fats and have a small quantity of carbohydrates, having a role in the immunity due to the high quantity of antibodies they contain. Gamma globulins are separated into three fractions through immunoelectrophoresis: γG globulins (IgG) , γA globulins(IgA) , γM globulins (IgM), these making up the proteic sublayer of many antibodies. Except the above mentioned proteins,plasma also contains other proteins, so in pathological states there could appear in the plasma some other proteins, such as: cryoglobulins, paraproteins, protein C reactive. The globulinic proteins determination is realized through the electrophoresis of the serum proteins on agar gel with the help of the CORMAY GEL PROTEIN 100 device.

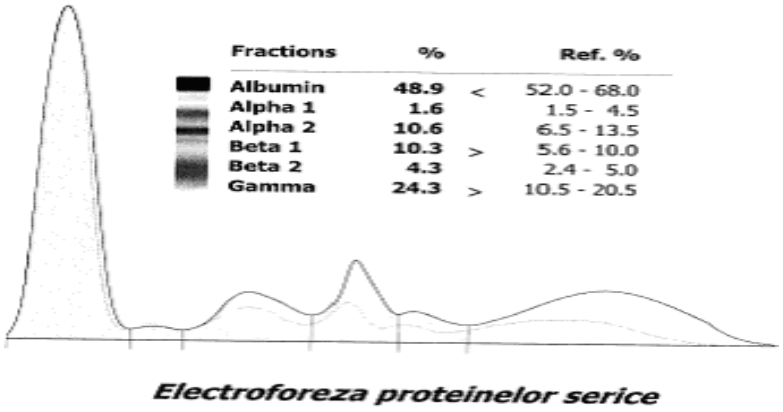


Figure 5: The electrophoresis of the serum proteins (original)

The albumins represent the biggest fraction of the plasma or serum proteins, they are formed in the liver, but small quantities have extrahepatic origins. They are more soluble than the other

fractions, homogenous, they could be obtain in crystalline state, and couldn't isolate subfractions through electrophoresis. They assure the water change between blood and tissues due to the colloid osmotic pressure, carry and fix different subtances (medicines, hormones), bound the water, ions and small molecules because they present on their surfaces equal anionic and cationic groups. The globulinic protein determination is realized through serum proteins electrophoresis on agar gel, with the help of the CORMAY GEL PROTEIN 100 device.

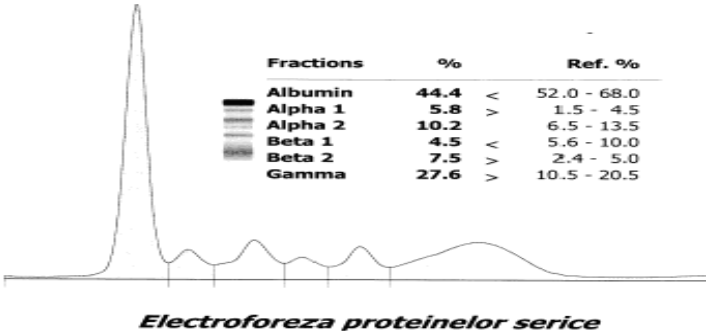


Figure 6: The electrophoresis of the serum proteins (original)

CORMAY GEL PROTEIN 100 is used for the electrophoretic separation of the serum proteins on agar gel, being obtained six proteic fractions: albumin, α_1, α_2, β_1, β_2, γ globulins. The serum proteins mofications were studied through the electrophoresis method on paper or agar, it is noticeable, among the diminishing of the albumins, the appearance in the γ globulin area of a high and narrow wave characteristic to the disease. These wave, indicating an homogenous increase of the proteins, could be placed in the area of γ globulins, the most frequent aspect, representing about 55% of cases : in „β" region 15,4%of cases , and rarely in the „α" region, representing 6,6 % of cases. The abnormal protein in the serum of the patients suffering of myeloma was called *paraprotein, myeloma globulin (M globulin) or component M (monoclonal component).*

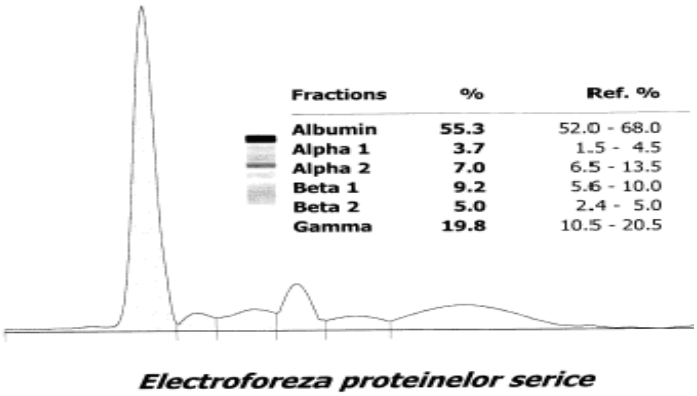


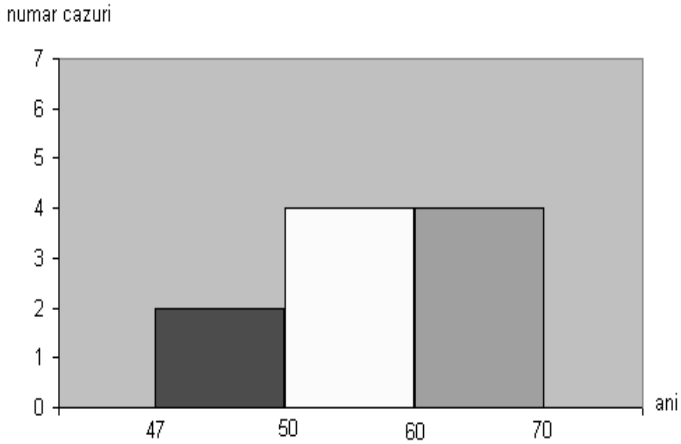
Figure 6: The electrophoresis of the serum proteins (original)

Normal plasmocytes produce immunoglobulins. The malign plasmocytes clone features capacity of analysis. Its products (myeloma proteins) are found at almost every patient suffering from multiple myeloma. Proteins, released by the myeloma cells are abnormal from a quantitative point of view, but not also from a structural one. When it’s about quantity, it could be got to values of 9 and 20g/100ml blood. Hyperproteinemia is given by the increase of the globulins. These proteins are identical from a chemical and structural point of view with the normal immunoglobulins. Myeloma globulins vary from a diseased to another, each sick person producing an unique protein, but its properties remain constant along the disease’ evolution. Applying immunoelectrophoresis has permitted the detection and classification of the myeloma proteins.Hyperglobulinemia explains the tendency of the erythrocytes to settle in rolls, the increase of the VSH (100-140mm/h), blood viscosity. Due to the dislocation of the normal plasmocytes from the myeloma ones, normal immnoglobulins are deducted.
A study made on the patients suffering from various affections pointed out a variation of the haematological parameters in terms of age, thus: related to gender, there have been found modifications both with the feminine gender and masculine one; related to age, there could be noted a higher variation of the biochemical indexes for age range between 47-70 years.

Figura 8: Genders repartition:

Number of cases	Feminine	Masculine
10	6	4

Figura 9:. The repartion of the cases according to age



The surveyed indeces were: the uric acid, calcium level, total proteins, pathologic proteins
Total proteins variation:
Figure10. Total proteins variation on male in multiple myeloma according to age

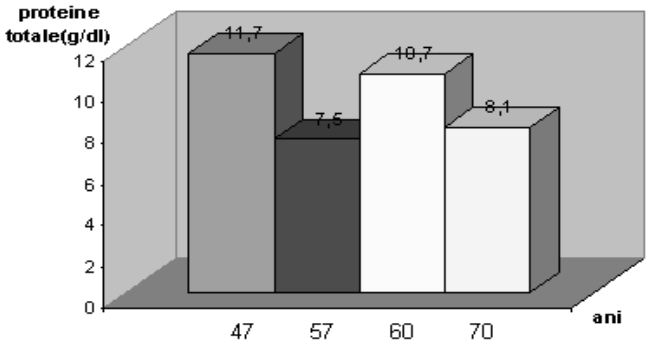
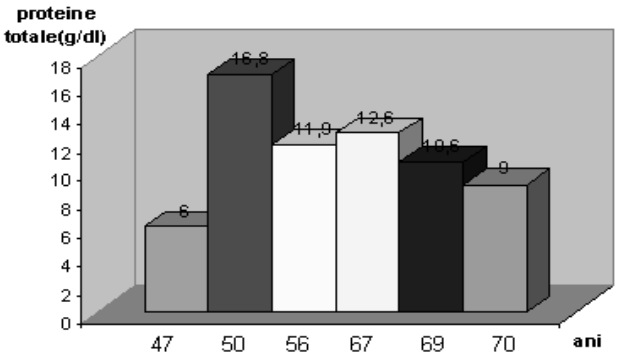


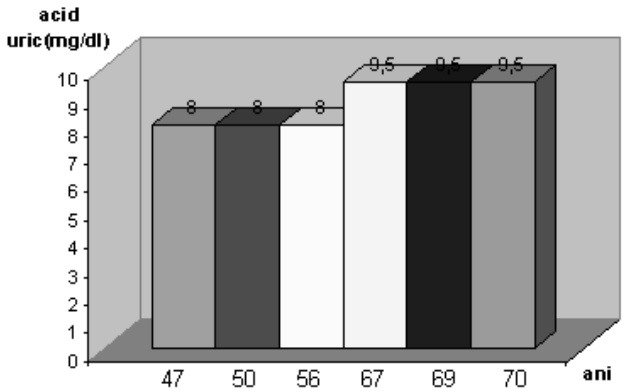
Figure 11: Total proteins on women within the multiple myeloma according to age



The proteins normal values are comprised between 6.3-8.2g/dl. Thus, it has been noted that in most of the cases, the total proteins value are high, excepting two cases on men and one on women.

The uric acid variation

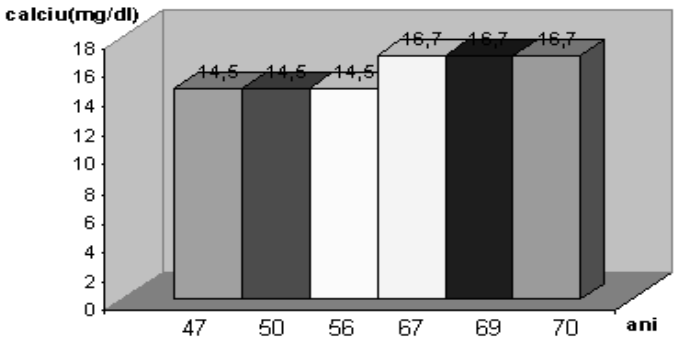
Figure 12: The uric acid variation on men and women within the multiple myeloma according to age



Within the multiple myeloma, the uric acid is frequent increased, and, especially in the cases with renal insufficiency. As it can be seen in the diagram, the normal values are between 2.5-7mg/dl.

Calcemia variation

Figure 13: Men and women calcemia variation within the multiple myeloma according to age



The normal values are between 8.4-10.2mg/dl. It has been found that in the case of the multiple myeloma there is a hypercalcemia, so it can be seen in the diagram. The values are not between the normal limits.

CONCLUSIONS

In the pathology of the cell system of immunity, a special group is constitute by the malign proliferations of the lymphocyte „B” cell system, such as: dominant proliferation pf the plasmocytes and hypersecretion of immunoglobulins, respectively plasma myeloma, various immnuolymphproloferations, with malign or border nature, Waldenstron disease, heavy chains disease, mediterranian lymphoma, plasma immunoblastoma.

Multiple myeloma is one of the diseases of the lymphoplasma system. The spectre of these diseases is very wide and it ranges from the monoclonal gammopathies, with or without any sign of disease, to the lymphoproliferative diseases with abnormal production of proteins.

Multiple myeloma is a disease world wide spread, without ethnic or geographic differences regarding the frequency. In the earlier statistics, it has been stated the predominance on men, nowadays, the incidence on genders tends to equalization.

The affection is known under varius names: plasma cell myeloma, plasmacytoma,plasmacytosis, Kahler-Rutizki disease, malign plasmocytosis, myelomatosis.

Patients suffering from multiple myeloma show: bleeding and coagulation disorders, hemolitic anaemia, and very rarely hyperlipoproteinemia – this could be sometimes accompanied by xanthomas, an din other diseases developing with hypergammaglobulinemia.

The production of light chains excessively jeads to their appearance in the urine, causing the Bebce-Jones proteinuria .

On the quantitve determination of the immunoglobulins, a characteristic feature is the increasing of a class of immunoglobulins and diminishing of the other classes.

The electrophoretic aspect is characterized by the presence of a haste abnormal bow, thickened and deformed in” boat’, making a difference between the immunoglobulins monoclonal growth and a polyclonalone one.

In general, 80%of the myeloma are of G immunoglobulin type; 15% of A immunoglobulin type; 1% of D and E immunoglobulin type.

There are cases where, the monoclonal component doesn't show in the serum – it is the case of the multiple myeloma, where the other tumors secrete only light chains, these being though presented in the urine.

The hypersecretion of myeloma proteins has been reported at all the patients presented: 9 cases have signaled growths of IgG, and only one case with growth of IgA, every time with a decrease of IgA and IgM, respectivelly IgG and IgM.

The serum electrophoresis – shows the presence of a narrow monoclonal band with mobility, varying from the γ to αregime, this being caused by the class of immunoglobulins that prolipherates. About 80% of myeloma reports paraprotein in the serum.

In the urine, light chains of immunoglobulins appear in 20-60% of cases., so that 98-99% of cases of multiple myeloma have signalled a paraprotein in the serum and/or urine.

Immunoelectrophoresis has pointed out in all the cases the monoclonal component, discrete or intense, and the total proteins have been found in all of the ten surveyed cases between 6g% and 16.8%, 7 cases having high values over 9%.

Also, the paraprotein from the D immunoglobulin myeloma is emphasized only in 1/3 of cases, because the D immunoglobulin myeloma contains, as a rule, from „λ" light chains, their presence in the urine have to raise the suspicion of D immunoglobulin myeloma.

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