USE OF PROTEINOGRAM: KEYS FOR DEMAND OPTIMIZATION

Serum proteinogram is the main method for detection and monitoring of monoclonal components (MC) in patients with suspected or confirmed monoclonal gammopathy (MG). The median age at diagnosis of MG is 65 years, with an overall incidence of 65 cases/100,000 population/year in those over 60 years of age, and 5 cases/100,000 population/year in those under 60 years of age. Due to the marked difference in incidence according to age, the Clinical Laboratory Societies indicate that it is not necessary to request this test in patients <50 years of age if there is no clinical suspicion of MG or to screen for MG in the general population, as there are no data to support that doing so improves patient outcome.

From a cost-effectiveness point of view, the proteinogram should not be used as a screening tool for specific protein changes and should not be repeated at intervals of less than one year if the electrophoretic pattern is normal in an initial study and no clinical or analytical changes related to the diagnosis of MG have occurred.

However, in clinical laboratory practice we encounter many requests for a proteinogram in contexts other than MG screening.

➢ Monoclonal Gammopathies

Monoclonal gammopathies (MG) are characterized by the expansion of a single clone of mature B lymphocytes (plasma cells and lymphoplasmacytes), which give rise to a monoclonal immunoglobulin called paraprotein or monoclonal component (MC). This MC is characterized by a single type of immunoglobulin heavy chain (γ in IgG, α in IgA, μ in IgM, δ in IgD and ε in IgE) and a single type of light chain (κ or λ).

MG has an approximate incidence of 45-60 cases per million population per year in Europe. In 70% of cases, it is an incidental laboratory finding without associated malignant B-lymphoid proliferation, known as Monoclonal Gammopathy of Uncertain Significance (MGUS). In the remaining 30% of cases, MC is associated with a neoplastic B-cell lymphoproliferative process, such as Multiple Myeloma (MM), Waldenström's Macroglobulinemia, lymphoma or Amyloidosis (**Table 1**).

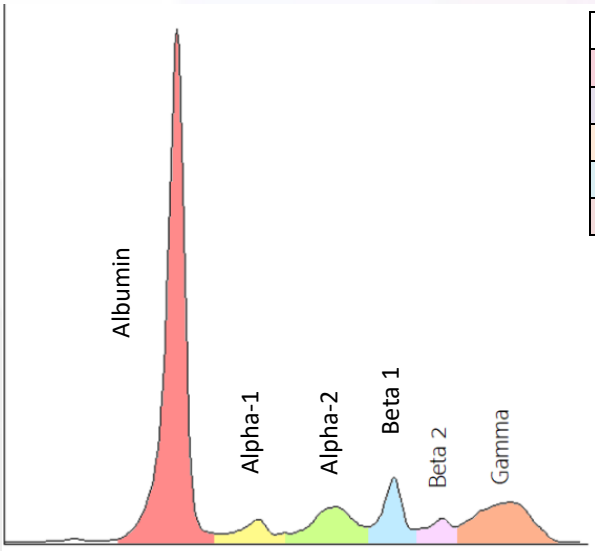
Monoclonal Gammopathy of Uncertain Significance (MGUS)
GMSI (IgG, IgA, IgM, serum free light chains κ or λ)
MGUS associated with neoplasms or processes not recognized as producing a monoclonal component
MGUS type Bence Jones idiopathic proteinuria

Malignant monoclonal gammopathies	
Multiple myeloma (IgG, IgA, IgM, free κ or λ light chains, Bence Jones)	Multiple myeloma (IgG, IgA, IgM, free κ or λ light chains, Bence Jones)
	Sintomático, progresivo o abierto
	No secretor
	IgD
	Osteoesclerótico (síndrome de POEMS)
	Leucemia de células plasmáticas
Solitary plasmacytoma	Solitary plasmacytoma
	Extramedular
Waldenström's Macroglobulinemia	
Light chain deposition disease	
Heavy chain disease	
Primary light chain amyloidosis	

***Table 1**: Classification of Monoclonal Gammopathies (MG)*

➢ Serum Proteinogram

In Catlab, the proteinogram is performed by capillary electrophoresis, an analytical method that separates serum proteins according to their charge/size ratio and then measures the absorbance of their peptide bonds at 200 nm. Finally, the spectrophotometric reading, which is proportional to the number of peptide bonds, is converted into a graphical signal called proteinogram, which is divided into different protein fractions (**Figure 1**).



Fraction	Ref. %	Ref. g/L
Albumin	55.8 - 66.1	36 - 56
Alpha-1 globulin	2.9 - 4.9	1.9 - 4.2
Alpha-2 globulin	7.1 - 11.8	4.6 - 10
Beta globulin	8.4 - 13.1	5.1 - 11.6
Gamma globulin	11.1 - 18.8	7.2 - 16

***Figure 1:** Electrophoretic profile of the proteinogram showing the different protein fractions in % and g/L.*

The different fractions of the proteinogram are quantified in % and their concentration in g/L is calculated from the total serum proteins.

If a suspected MC is detected in the proteinogram, appearing as a 'peak' or narrow band, it is characterized by immunofixation and/or immunosubstraction, which allows the determination of the MC isotype using specific antibodies against the heavy chains (IgG, IgA, IgM, IgD, IgE) and light chains (kappa and lambda) (**Figure 2**).

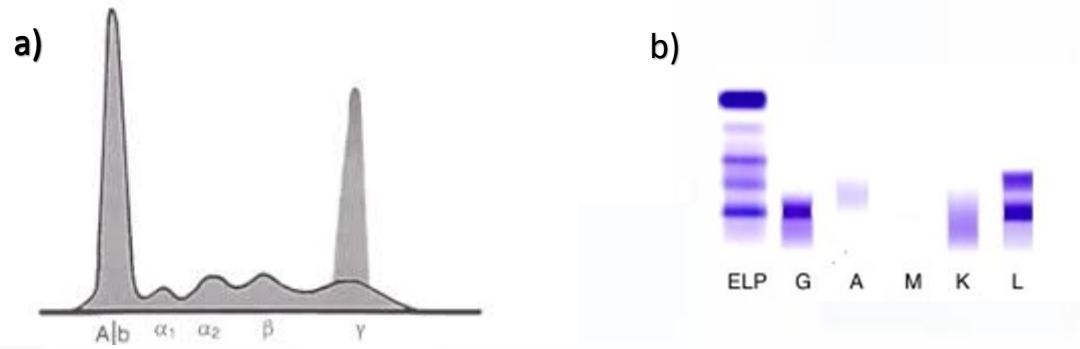


Figure 2: a) *Proteinogram showing a monoclonal band in the gamma region and* ***b)*** *immunofixation showing the presence of monoclonal IgG lambda component and monoclonal. lambda free light chains.*

It is important to note that the diagnosis of MG is integrated and that the result of the proteinogram performed at the Immunology department must be interpreted together with other parameters and tests performed in other laboratory services: Biochemistry (creatinine and glomerular filtrate, calcium, total proteins, albumin, LDH, B2 microglobulin, acute phase reactants); Hematology (hemogram); Flow cytometry (immunophenotyping of neoplastic cells); Genetics (cytogenetic alterations of neoplastic cells) and other clinical services: Hematology, Internal Medicine and Primary Care, mainly.

➢ Reviewing the demand for proteinograms in our laboratory

We retrospectively analyzed the data of proteinograms performed in our laboratory in 2023 from the health area of the Hospital Universitari Mutua de Terrassa, Hospital de Terrassa, Hospital Fundació Sant Joan de Déu de Martorell and ICS Vallès Occidental Primary Care, areas with a population of approximately 820,000 inhabitants.

A total of 22,625 proteinogram requests were collected, with an average patient age of 63 years. **Table 2** and **Figure 3** show, by age group: the number of proteinograms requested, the number of proteinograms in which a MC was detected and the ratio % MC / age group.

23% of the requests (n=5,226) were for patients under 50 years of age. The age groups with the highest number of proteinogram requests were 70-79 and >80 years.

Age group	Nº of proteinograms	Nº of proteinograms with MC	% MC / age group
0-9	60	0	0,00%
10-19	420	0	0,00%
20-29	900	2	0,22%
30-39	1312	17	1,30%
40-49	2534	91	3,71%
50-59	3660	331	9,07%
60-69	4214	579	13,74%
70-79	5103	1055	20,67%
>80	4427	946	21,37%

***Table 2**: Representation by age group of the number of proteinograms requested in 2023, nº of proteinograms with MC and % of MC detected by age group.*

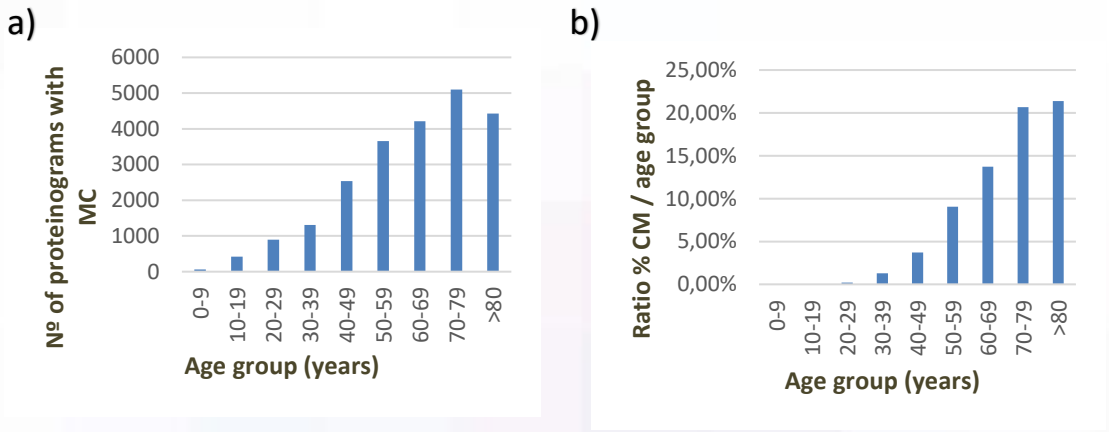


***Figure 3**: **a)** Nº of patients studied by age group and **b)** % of MC detected out of the total number of patients by age group.*

The presence of MC was detected in 13.3% of proteinograms (n=3,021). The age group with the highest percentage of MC detection was >80 years. In patients <50 years, the presence of MC was detected in 110 cases (0.5% of all proteinograms). Of these, 70 were requested by Hematology and 40 by other services. Of these 40, 35 correspond to transient MGUS or MC, 4 are MG follow-ups and one was added by the laboratory itself when plasma cells were detected in the hemogram, thus diagnosing MM.

According to our data, age >50 years was obtained as a cut-off point for MC screening with a sensitivity >95% (AUC 0.687; CI 0.681-0.693), in accordance with the described recommendations.

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Proteinogram ordering recommendations

- The use of the proteinogram is only useful in the diagnosis and/or follow-up of MG.
- It is not recommended to request a proteinogram in patients <50 years of age unless there is a clinical suspicion of MG.
- If the electrophoretic pattern is normal, it should not be repeated before one year has elapsed, unless there are clinical or analytical changes that warrant it.
- Since the proteinogram does not provide individual information on a specific protein, it is recommended that the concentration of isolated proteins in serum is studied using the specific quantification method for each protein. It should not be used as a tool to assess nutritional status.

Laura García Bravo

Immunology Department

Núria Pacheco

Clinical Analyses Department

Mireia Fonolleda

Immunology Department

CATLAB

www.catlab.cat

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