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ORIGINAL ARTICLE

# A retrospective study of the relative utility of electrophoresis in the investigation of serum proteins

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## ABSTRACT

**BACKGROUND**
Serum protein electrophoresis (SPE) is widely used for diagnosis, management and monitoring of different immune disorders. Serum protein alterations provide valuable insights about these disorders. Electrophoresis separates proteins based on their physical properties, and the subsets of these proteins are used in interpreting the results. The purpose of this study was to analyze the results of pathological and non-pathological SPE of Ecuadorian patients.

**METHODS**
A retrospective observational study was conducted using data of pathological (871 samples) and non-pathological (752 samples) serum protein electrophoresis data of 1623 patients who attended the Gamma Clinical Laboratory of Portoviejo, province of Manabi, Ecuador. Bivariate and exploratory factorial analysis were done using following parameters: age, sex, survival, laboratory diagnosis according to electrophoretogram.

**RESULTS**
The highest frequency of deaths occurred in females (52.9%). The most frequent pathological outcomes were polyclonal hypergammaglobulinemia (49.5%), acute inflammation (21.1%), and monoclonal gammopathy (14.70%). Exploratory factorial analysis revealed that the results of pathological SPE were mainly influenced by albumin and γ-globulin fractions followed by α1-globulin, α2-globulin, and β1-globulin-β2-globulin behavior that differed from non-pathological SPE. The Kaiser-Meyer-Olkin measure was considered adequate for data analysis, and Bartlett's test of sphericity was highly significant ($p<0.001$), corroborating that factor analysis is useful for the data set.

**CONCLUSIONS**
This evidence warrants the need for rigorous analysis of globulin concentration in SPE to avoid biased diagnosis based solely on visual inspection of patterns in the electrophoretogram. Special attention should be paid to the relationships between fractions that show the greatest influence on SPE results.

**Keywords**: Blood protein electrophoresis; hypergammaglobulinemia; monoclonal gammopathy; exploratory factorial analysis.

**Abbreviations**
SPE: Serum protein electrophoresis
MG: Monoclonal gammopathies
MM: Multiple myeloma
MGUS: Monoclonal gammopathy of uncertain significance
AMM: Asymptomatic multiple myeloma
PG: Polyclonal gammopathies
HyperG: Polyclonal hypergammaglobulinemia
AI: Acute inflammation
NS: Nephrotic syndrome
HC: Hepatic cirrhosis
CI: Chronic inflammation
HypoG: Hypogammaglobulinemia
BG: Biclonal gammopathy
OG: Oligoclonal gammopathy
PCs: Principal components
KMO: Kaiser-Meyer-Olkin
RI: Reference interval

## INTRODUCTION

Serum protein electrophoresis (SPE) is an analytical method that separates proteins into fractions known as albumin, α-1 globulin, α-2 globulin, β-1 globulin, β-2 globulin, and γ-globulin.[1,2] The interpretation of the signals or patterns of these fractions in the electrophoretogram is often used as a screening procedure in the identification of the protein profile for a first diagnosis according to the characteristics shown in the presented regions.[3–6] In healthy individuals, the signals of these fractions present symmetrical bell-shaped curves [7] and if there is any pathology, some asymmetry can be observed with increased or decreased signal intensity due to the presence of various proteins depending on their migration site.[6–8]

SPE is the most widely used technique to study the serum protein profile due to its ease of use, speed of analysis,[5,8] and efficacy for the diagnosis and follow-up of patients with suspected or diagnosed monoclonal gammopathies (MG) such as multiple myeloma (MM), Waldenström's macroglobulinemia, and primary amyloidosis. In addition, SPE is very useful for observing dysproteinemias that usually occur, among others, in autoimmune diseases, cancer, renal diseases, and immunodeficiencies.[2,7,9–14] These diseases are currently increasing in the population [12,15] possibly associated with the increase in environmental pollutants, such as agrotoxins.[15]

The diagnosis of MG presents a challenge in biomedical sciences because both malignant and non-malignant MG can be found in this group, and both are incurable.[2,9] Within the category of non-malignant MG, monoclonal gammopathy of uncertain significance (MGUS)[12,16] corresponds to 60% of diagnosed cases.[17] This evolves to a malignant phase called asymptomatic multiple myeloma (AMM).[12,18] On the other hand, in the category of malignant MG is to be found MM, the second most frequent hematological neoplasm with approximately 15% of diagnosed cases.[19] According to recent estimates, worldwide, 159,985 new cases of MM are diagnosed annually.[20] In the United States, 32,110 new cases[19] are diagnosed each year and in Spain, more than 2,000 new cases. [21] In Ecuador, Vélez et al.[22] analyzed data from 629 cases of pathological SPE, of which 53% corresponded to MG with chronic inflammatory patterns, 17% other pathologies, 14% MG (mostly MM), 12% acute inflammation, and 4% hypogammaglobulinemia. SPE alterations were predominant in females with a mean age of 63 years. Weak monoclonal and oligoclonal components were found that were not related to the diagnosis of MG, which components emphasized the importance of the interpretation that should be given to the electrophoretogram in cases of weak monoclonal components to avoid bias in the timely diagnosis of potentially fatal MG. Similarly, Talledo et al.[23] analyzed the data

of 237 SPE of patients with diagnosed MG who attended an oncological hospital in the province of Manabí. These investigators found that 29% presented chronic inflammatory patterns, 12% acute inflammation, 12% isolated abnormal patterns of non-gammaglobulin fractions, 10% polyclonal hypergammaglobilinemia (HyperG), 3% hypogammaglobulinemia, 1% liver disease, and 14% MG, of which 53% corresponded to MM and 12% to non-secretory MM. Male patients with a mean age of 64 years were the most affected by these two diseases.

On the other hand, total protein concentration can be affected by both biological and analytical phenomena.[16,18,24–31] Among the biological ones can be mentioned hyperproteinemia (increased circulating protein mass due to mono- or HyperG), hemoconcentration due to fluid loss (e.g., diarrhea, vomiting, heat stroke), hypoproteinemia (decrease in circulating protein mass due to malnutrition or renal losses, and hemodilution (due to increased vascular water or hydraulic overload).[8,13] Regarding the analytical phenomena, electrophoretic mobility can be affected by the size, charge, and strength of the applied electric field, by pH and buffers, or analytical interferences can appear such as those generated by the occurrence of hemolysis in the sample or of fibrinogen due to the use of anticoagulated blood.[18,27,32] In addition, it should be noted that it is not in all cases that a normal protein profile is obtained, signifying the absence of a monoclonal component, which may not be detected because its concentration is below the detection limit of the instrument used and requires more sophisticated techniques with higher resolution.[6,24]

Recent studies report the use of patterns observed in the SPE bands for the diagnosis, management, monitoring, and epidemiology of some hematological diseases, MG, MM, liver diseases, renal diseases, and autoimmune disorders. In contrast, our study covers epidemiological aspects and explores the dataset of pathological and non-pathological SPE using classical and multivariate statistical techniques to uncover underlying behaviors and the contribution of SPE fractions to the diagnosis of pathologies or conditions that cause alterations in serum proteins. In this regard, a retrospective observational study was conducted to analyze the results of pathological and non-pathological SPE of Ecuadorian patients.

## METHODS

### Research design

A retrospective observational study was conducted based on the analysis of pathological and non-pathological SPE data of patients seen at the Gamma Clinical Laboratory (Synlab Group) in Portoviejo, Manabí Province, Ecuador, from January 2015 to December 2020.

### Study subjects

The population consisted of 2144 SPE tests performed between January 2015 and December 2020 at the Gamma Laboratory (Synlab Group) in Portoviejo, Ecuador. A non-probabilistic sampling method was applied to select only the first SPE result of each patient, yielding a sample of 1623 cases. Of these, 871 patients had pathological SPE results, and 752 patients had normal SPE results, based on the pattern observed in the electropherogram bands. Patients of both sexes and all ages were selected if their laboratory database records included concentrations for each fraction and the laboratory diagnosis according to the pattern observed in the electropherogram bands.

### Laboratory analysis

SPE was performed using a Sebia MINICAP FLEX-PIERCING capillary electrophoresis system. The clinical indications were broadly categorized into: age, gender, survival, laboratory diagnosis according to the pattern of signals in the electrophoretogram [polyclonal hypergammaglobulinemia (HyperG), acute inflammation (AI), monoclonal gammopathy (MG), nephrotic syndrome (NS), hepatic cirrhosis (HC), chronic inflammation (CI), hypogammaglobulinemia (HypoG), biclonal gammopathy (BG), and oligoclonal gammopathy (OG)], and the concentration of each fraction of the electrophoretogram (albumin, α1-globulin, α2-globulin, β1-globulin, β2-globulin and γ-globulin) expressed in percentage. Data on deceased patients were obtained from the website of the Civil Registry of Ecuador up to February 2020, to avoid bias due to COVID-19 deaths during 2020.

### Statistical analysis

Using IBM® SPSS® Statistics, version 25, the data mining protocol[33] began with the exploratory analysis of the data to evaluate the characteristics of the distribution of the variables using the Kolmogorov-Smirnov test, which

indicated that the scalar variables did not follow a normal distribution ($p<0.05$). To perform data transformations, different methods were applied, such as operations of square root, the inverse of square root, quadratic hyperbolic, logarithms (e.g. cubic, natural, decimal, the inverse of the decimal logarithm), the fourth power inverse, and general power transformations (Box-Cox and Yeo-Johnson). The Anderson-Darling test for normality of data was applied but failed to find normal data distributions. In all tests, a $p<0.05$ was considered statistically significant. Results were expressed as absolute and percentage frequencies and numerical characteristics were based on measures of location (median, minimum and maximum, and quartiles) and range as a measure of dispersion. The relationship between nominal variables was determined by using contingency tables and Pearson's $\chi^2$ test was used as a hypothesis test. The relationship between the SPE fractions was verified by Spearman's Rho correlation test[34] considering the degree of association according to the linear scale: very weak (>0.00-0.20), weak (0.20-0.40), medium (0.40-0.60), strong (0.60-0.80) and very strong (0.80-1.00), as well as the direction (positive sign indicating direct proportion and negative sign indicating inverse proportion).[35] Repeated Measures One-way ANOVA and One sample t test were performed using GraphPad Prism 9.0.0. software.

The exploratory factor analysis technique with the principal component extraction method was applied. This multivariate statistical method reduces the dimensionality of the data set to a new set of variables, known as principal components (PCs) with minimal loss of information. PCs are obtained as a linear combination of the original variables, which retain the higher variance of the data in a new and lower dimensional space.[33,36,37] In this case, the concentrations of pathological and non-pathological SPE fractions (albumin, α1-globulin, α2-globulin, β1-globulin, β2-globulin and γ-globulin) were used. The PCs matrix was obtained using the Varimax rotation method, based on orthogonal rotation that reduces the number of variables that have high loadings on each factor.[33] The PCs that presented eigenvalue >1 and that explained at least 70% of the total accumulated variance were selected.[37] The relevance of the application of factor analysis to the set of variables studied was verified by means of the Kaiser-Meyer-Olkin (KMO) measure, considering values between 0.50 and 1.00 for the existence of adequacy of the data to the factor analysis model, and values between 0.00-0.50 for the non-existence of adequacy of the data to the factor analysis model. On the other hand, the adequacy of the data to the factorial model was corroborated by applying Bartlett's test of sphericity, which determines whether or not the correlation matrix is an identity matrix that would indicate that the factorial model is inadequate.[33] This test was applied using the $\chi^2$ test with a significance level of $\alpha=0.05$. If the $p<0.05$, the factorial model is considered adequate for the data set.

**Ethical aspects**

The database provided by the Gamma Laboratory (Synlab Group) of Portoviejo was anonymized. Ethical criteria were met in accordance with the Declaration of Helsinki, maintaining confidentiality of information, beneficence, and non-maleficence. In addition, the Bioethics Committee of the Universidad Técnica de Manabí (Portoviejo, Ecuador) approved the study (Approval code 21-10-021).

## RESULTS

**Sample characteristics**

The numerical characteristics of the SPE dataset of 1623 patients who attended the Gamma Laboratory (Synlab Group) in Portoviejo, Ecuador between 2015 and 2020 were presented in two categories (Figure 1): (i) pathological SPE (871; 53.66%) and (ii) non-pathological SPE (752; 46.33%). The female patients with pathological SPE predominated (53.84%, 469) over the male patients (46.15%, 402). The mean age showed significant differences ($p=0.03$), being 55.58 ± 20.24 years and 58.03 ± 20.25 years for the females and males, respectively. The age range was between 1 and 93 years for females and between 1 and 92 years for males. Twenty five percent (quartile three) of the female patients was aged ≥70 years and that of the male patients was ≥ 73 years. It is noteworthy that 10% (quartile one) of this sample presented an age below 45 years for the females and 48 years for the males. Survival data showed no significant differences, for the total number of deceased with respect to those alive ($p=0.451$). The highest frequency of deaths was found in females (52.90%, 287) compared to males (47.10%, 256).

Similarly, in patients with non-pathologic SPE, female patients predominated (58.50%, 440)

with respect to male patients (41.50%, 312), but showed no significant differences in mean age (p=0.122), being 51.92 ± 18.81 years and 49.03 ± 21.19 years for females and males, respectively (younger age than in patients with pathologic SPE). The age range was between 1 and 93 years for the female sex and between 1 and 92 years for the male sex. In both genders, 25% (quartile three) of the patients was aged ≥65 years (younger than in patients with pathologic SPE). Nonetheless, survival data showed no significant differences for the total number of deceased patients with respect to those alive. In this group (p=0.382), it is noteworthy that 56.10% (422) of the patients died even though they had a normal diagnosis of progression-free survival. Of these, 57.10% (241) were female and 42.90% (181) were male.

More than half of the SPE results analyzed (871; 53.66%) were pathological. This could be due to the fact that in this environment, where the research was carried out, the SPE test was requested when dysproteinemia was found in routine examinations or due to clinically suspected MG. The most frequent diagnoses in decreasing order were as follows: HyperG > AI > MG > NS > HC > CI > HypoG > BG > OG (Figure 2). HyperG was primarily diagnosed in females (59.60%; 257) as compared with males (40.40%, 174), AI was slightly higher in males (51.60%, 95) than in females (48.40%, 89), whereas MG was mainly in males (56.30%, 72) compared to females (43.80%, 56). The diagnoses associated with the pathologies that presented the highest frequency of mortality were HyperG (59.90%, 258), AI (63.00%, 116), MG (71.90%, 92), and HC (86.70%, 26).

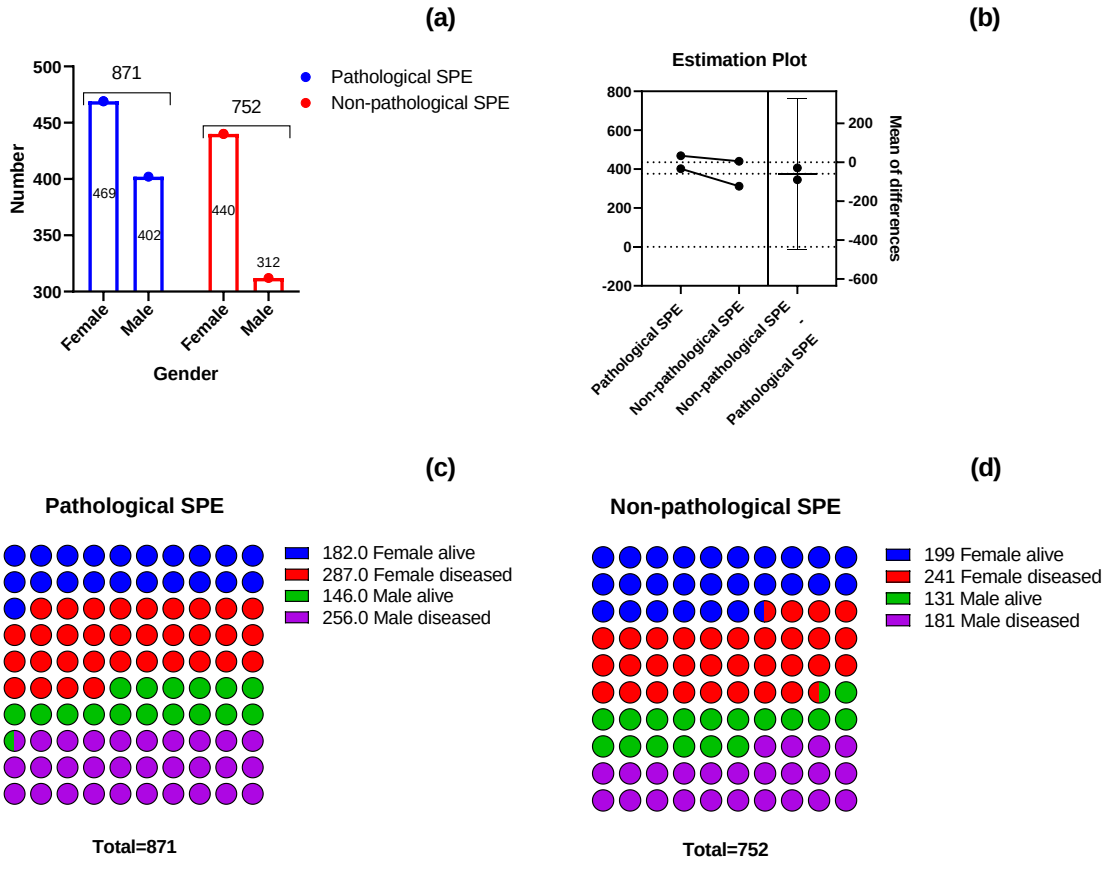


**Figure 1**. Disease overview. Distribution by gender (a), estimation plot (b), survival of patients with pathological (c) and non-pathological SPE (d)

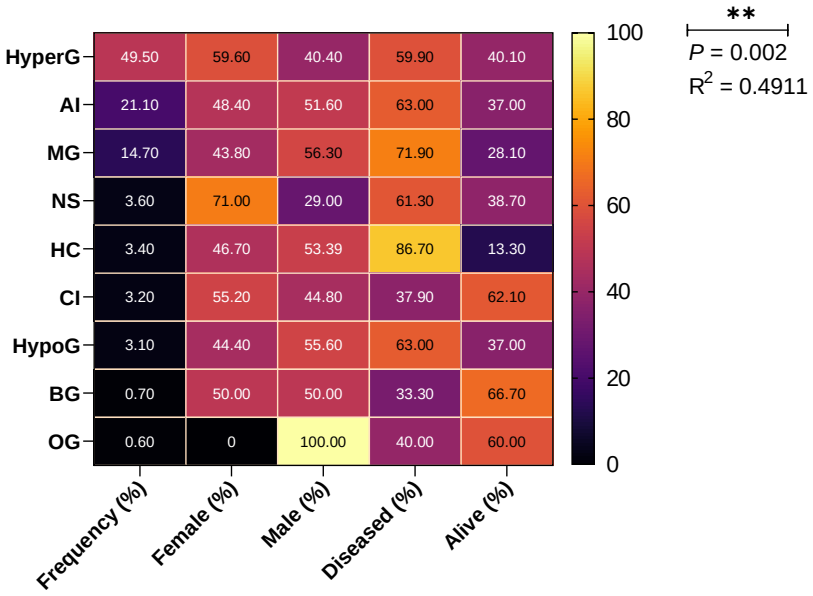


**Figure 2**. Laboratory diagnosis according to gender and survival of patients with pathologic SPE (** = Repeated Measures One-way ANOVA). HyperG: Polyclonal hypergammaglobulinemia, AI: acute inflammation, MG: Monoclonal gammopathies, NS: Nephrotic syndrome, HC: hepatic cirrhosis, CI: Chronic inflammation, HypoG: Hypogammaglobulinemia, BG: Biclonal gammopathy, OG: Oligoclonal gammopathy

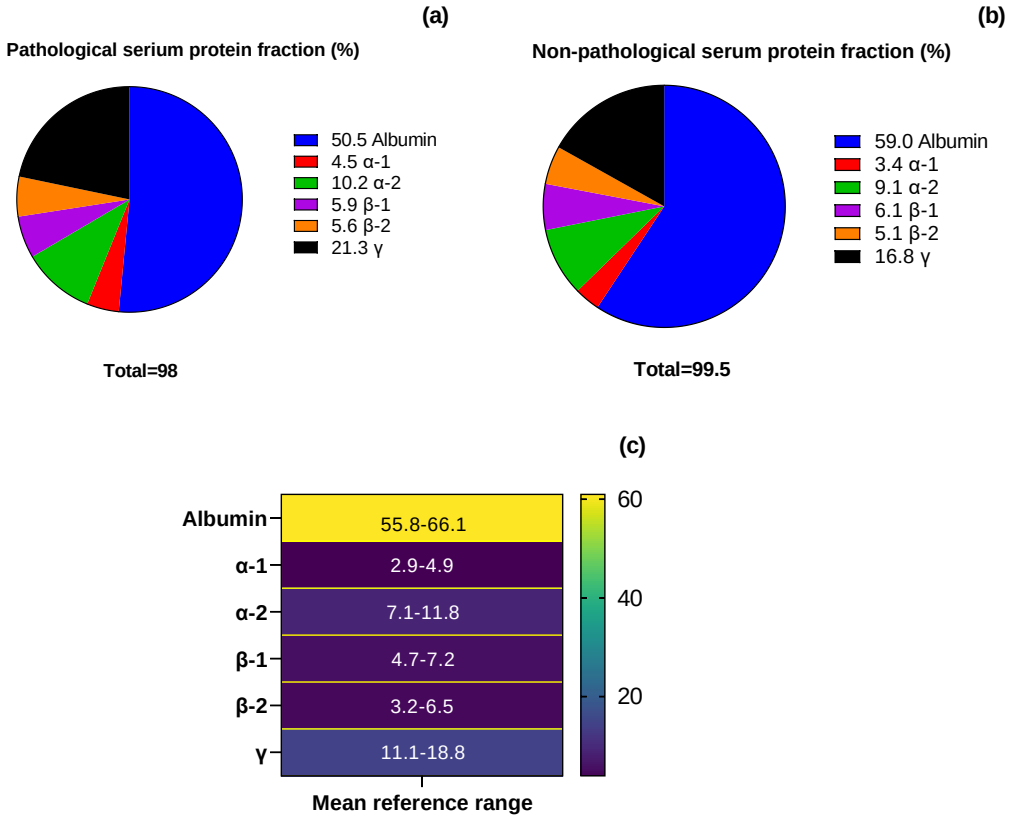


**Figure 3.** Concentration of (a) pathological, (b) non-pathological SPE fractions and (c) reference range. [α1: α1-globulin, α2: α2-globulin, β1: β1-globulin, β2: β2-globulin and γ: γ-globulin fractions]

**Bivariate statistical analysis**

The value of the SPE test lies in its ability to provide a detailed profile of the serum protein composition, which can be crucial for early diagnosis and management of certain diseases. Therefore, it is a valuable diagnostic tool in various medical disciplines where the traditional interpretation of SPE has been based on the density and position of these bands for each protein fraction. As such, it is important to explore the dataset for possible correlations and latent associations among the fractions using statistical tools. In this regard, with the purpose of understanding the possible relationships, strength, and direction of the association between the fractions of pathological and non-pathological SPE, the Spearman's Rho correlation test was applied to the dataset.

The behavior of each fraction in pathological and non-pathological SPE was assessed by bivariate relationships using Spearman's nonparametric Rho test (Table 1) as a measure of linear association between the concentration ranges of SPE fractions.[34,35]

In pathological SPE, the albumin/γ-globulin ratio stands out with a highly significant correlation and strong association in inverse proportion ($\rho$=–0.700, $p<0.001$), i.e. when albumin concentration decreases, γ-globulin concentration increases. Similarly, a highly significant correlation was obtained between α1-globulin/α2-globulin fractions ($\rho$=0.698; $p<0.001$) in direct proportion. In non-pathological SPE, the trend was similar to that observed in pathological SPE, i.e. the correlation between albumin/γ-globulin fractions was highly significant ($\rho$= –0.660; $p<0.001$) and constituted a strong association in inverse proportion, whereas for α1-globulin/α2-globulin fractions the correlation was significant in direct proportion ($\rho$=0.550; $p<0.050$).

**Exploratory factorial analysis**

A factor analysis was performed on the pathological and non-pathological SPE data sets to obtain principal components (PCs) considering the results of the albumin, α1-globulin, α2-globulin, β1-globulin, β2-globulin and γ-globulin fractions (Figure 4). From the pathological SPE data set, three PCs were obtained that explain 84.76% of the variance of the original variables, i.e. the variability caused by alterations in the concentrations of albumin and globulin fractions due to some disease. PC 1 indicated that the fractions with the greatest influence on the results of an SPE are albumin (0.963) and γ-globulin (–0.901). Both present high values (close to 1) and with opposite signs demonstrating the strong relationship between both fractions. On the other hand, PC 2 showed that the α1-globulin (0.909) and α2-globulin (0.905) fractions were strongly correlated and had an important contribution to the SPE results, while in PC 3 the β1-globulin (0.630) and β2-globulin (0.894) fractions, although presenting lower values than the rest of the fractions, also had an important contribution and were correlated. The data set of the non-pathological SPE showed a different behavior from that of the pathological SPE. In this case, two PCs were obtained that explained 92.66% of the variance of the original variables. PC 1 indicated that the albumin fraction (– 0.958) had the greatest influence followed by β1-globulin (0.607) and β2-globulin (0.722), and in PC 2, the greatest contribution was presented by α1-globulin (0.715), α2-globulin (0.782) and γ-globulin (–0.729). In this way, multivariate statistical techniques are used to demonstrate the differences in the behavior of the fractions in the electrophoretogram and their influence on the results, which could not be obtained with bivariate statistical techniques.

The factor analysis was verified using the KMO test, which indicates the usefulness of the data for detecting the behavior of the relationships, and the proportion of the variance in the variables that may be caused by unknown factors. In this sense, KMO=0.396 and 0.448 were obtained for the pathological and non-pathological SPE, respectively, which were considered adequate, although they are below the accepted interval (0.500-1.000) for considering a factor analysis to be useful. Possibly, the measurement was affected by the heterogeneity of the samples and by the fact that these are results from biological samples, which makes these analyses more complex. Additionally, Bartlett's test of sphericity for pathological SPE ($\chi^2$=5242.736; $p<0.001$) and non-pathological SPE ($\chi^2$=6621.065; $p<0.001$) was significant, corroborating the fact that the applied factor analysis was useful for the data set analyzed.

Table 1. Spearman's Rho correlation of pathological and non-pathological SPE fractions

| Pathological SPE | | | | | |
|---|---|---|---|---|---|
| **Globulin** | **Albumin** | **Globulin** | | | |
| | | **α1** | **α2** | **β1** | **β2** |
| α1 | –0.262 | | | | |
| α2 | –0.083 | **0.698**** | | | |
| β1 | 0.200 | 0.082 | 0.169 | | |
| β2 | –0.302 | 0.162 | 0.116 | 0.326 | |
| γ | **–0.700**** | –0.296 | –0.506* | –0.385 | 0.016 |
| **Non-pathological SPE** | | | | | |
| **Globulin** | **Albumin** | **Globulin** | | | |
| | | **α1** | **α2** | **β1** | **β2** |
| α1 | –0.292 | | | | |
| α2 | –0.314 | 0.550* | | | |
| β1 | –0.383 | 0.169 | 0.160 | | |
| β2 | –0.564* | –0.010 | 0.080 | 0.363 | |
| γ | **–0.660**** | –0.201 | –0.307 | –0.003 | 0.255 |

*Significant correlation p<0.05 **Highly significant correlation at p<0.001

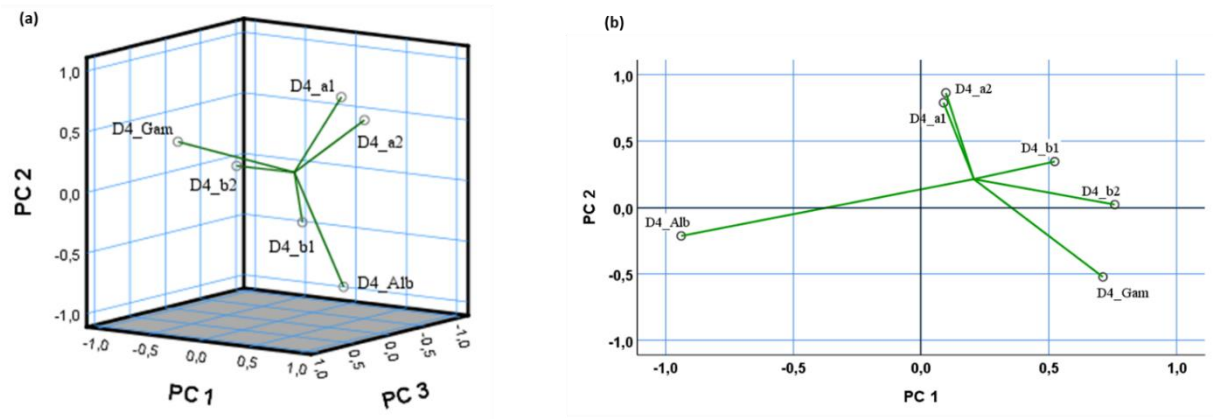


**Figure 4**. Principal components analysis for (a) pathological and (b) non-pathological SPE. [D4_Alb: Albumin, D4_a1: α1-globulin, D4_a2: α2-globulin, D4_b1: β1-globulin, D4_b2: β2-globulin and D4_Gam: γ-globulin fractions]
Note: "All commas in Figure 4 should be read as points (periods), eg. 0,5 becomes 0.5 etc."

## DISCUSSION

The study sample (Figure 1) consisted of more women than men, possibly explaining the higher frequency of pathological SPE outcomes and deaths among women. Shannon et al.[38] stated that the ways in which age and social determinants contribute to the health status of women compared to men vary between countries. In addition, the same investigation has indicated that studies comparing men and women are inadequate for explaining the processes involved in medical help- or assistance-seeking behavior when faced with a suspected illness. Importantly, "traditional male behavior" is an explanation for delays in seeking medical help or assistance on the part of men. On the other hand, Oksuzyan et al.[39] reported that men and women become ill in different ways, and it is women who come earlier for consultation when faced with the uncertainty of becoming severely ill. These studies highlighted the need to address the social structures, gender norms, and roles that differentially influence morbidity with aging in both sexes. In addition, advanced age increases the risk of chronic diseases. Aunan et al.[40] reported that advanced age is the most relevant risk factor for the development of cancer in general and for many types of cancer in particular. They further indicated that more than half of cancer diagnoses occur in people older than 70 years.

The results presented in Figure 2 show some similarity in the decreasing order of diagnostic frequency with that reported by Boban et al.,[32] which evaluated visually the pattern of signals in the γ-globulin region of the electrophoretogram and its correspondence with different clinicopathological states of 7259 patients aged 1 to 89 years who underwent SPE. Results indicated that 6.40% showed some alterations of the pattern in the γ-globulin region; the most prevalent findings of this study were HyperG (4.20%), followed by MG (1.40%) and HypoG (0.80%).

The highest frequency of pathological SPE corresponded to HyperG possibly because it is a polymorphic humoral immune reaction that causes increased concentrations of all immunoglobulins.[8,41] This anomaly has no precise pathological significance and its occurrence may be due to multiple factors of the individuals, such as geographical origin (especially from tropical countries), malnutrition, bacterial and parasitic infections (toxoplasmosis, leishmaniasis, malaria), viral infections (hepatitis, acquired immunodeficiency virus, Epstein-Barr virus), autoimmune diseases (Sjögren's syndrome, disseminated lupus erythematosus), sarcoidosis, viral-related liver conditions [8,16,18] or toxicity,[15] etc. It has also been reported that moderate to severe HyperG may reflect an underlying condition such as liver disease, connective tissue disease, hematologic disorder, inflammation, or malignancy. Moreover, it has been described that some patients with systemic lupus erythematosus showed hypoalbuminemia with HyperG as a consequence of lymphocyte hyperactivity characteristic of autoimmune diseases.[16,25] It is important to emphasize that in our study MG ranked third (14.70%) among the pathological SPE. These diseases involve a set of disorders associated with the uncontrolled proliferation of a clone of plasma cells that produce immunoglobulin molecules or fragments thereof known as monoclonal components.[16,25,26,42,43] The spectrum of MG diseases is very varied. According to Oliveros et al.,[44] the diagnosis of malignant MG can take from six months to a year and sometimes up to two years from the time the patient first goes to the doctor for presenting some symptomatology, which in many cases is non-specific, until a definitive diagnosis is obtained. In addition, the environment in which this work is carried out may also be influenced by the scarce local resources and the cost of the tests necessary for confirmatory diagnosis. This reality is a frequent cause of inefficient screening processes. Studies worldwide agree on establishing protocols for the detection, isotype identification, and follow-up of MG through the combined use of tests that should include an SPE, serum immunofixation, immunoglobulin quantification, and the blood-free light chain assay which will allow understanding of the importance of early detection and treatment to avoid unfavorable disease progression.[2–5,41,45]

The differences between pathological and non-pathological serum protein fractions (Figure 3) may occur because albumin concentration tends to decrease in abnormal conditions and globulins show a simultaneous increase or in some cases remain unaltered in the physiological response to inflammation, trauma, and myocardial infarction.[8] On the other hand, the time of onset and slow evolution of the disease can affect the concentration of proteins in serum, as indicated above.[44]

From Figure 3 it can be inferred that in pathological SPE there is an evident alteration of the albumin/globulin (A/G) ratio. This ratio should be higher than 1, a value lower than 1 may clinically indicate the presence of autoimmune, hepatic, or renal diseases.[2,9,13,16,26,43] According to the study by Vélez et al.,[22] MG showed higher average total protein concentrations; however, there were no significant differences between MG and HyperG in terms of total protein concentrations, but there were significant differences in albumin concentration and A/G ratio which were higher in HyperG than in MG (3.70 g/dL vs. 3.21 g/dL; 1.06 g/dL vs. 0.82 g/dL, respectively). Nevertheless, according to the above literature, it has been concluded that a total protein test result above the reference values would not be sufficient to differentiate between MG and HyperG; however, hyperproteinemia with hypoalbuminemia and an A/G ratio of less than 1 would be more indicative of a diagnosis of MG than of HyperG.

As shown in Table 1, the behavior of the correlation between the albumin/γ-globulin fractions of pathological and non-pathological SPE follows a similar trend. However, the degree of association of α1-globulin/α2-globulin is higher in non-pathological SPE. In this way, the bivariate statistical analysis evidences the relationship between the albumin/γ-globulin fractions, which coincides with the interpretations that have traditionally been made qualitatively by visual inspection of the patterns in this region of the electrophoretogram; however, the bivariate analysis is limiting in this sense, because it does

not allow distinguishing pathological and non-pathological SPE, so the application of more sophisticated statistical techniques that allow revealing behaviors that are not possible with the bivariate analysis is required. To verify these results, a multivariate analysis was applied using exploratory factor analysis.

The principal components analysis (Figure 4) demonstrated the need for critical analysis by the clinician in the interpretation of pathologic and non-pathologic SPE to avoid biased diagnoses. For non-pathological SPE that, on visual inspection of the signals in the electrophoretogram, show a normal behavior, it is very important to contrast it with the trend of the quantitative results of each globulin, that is, to observe the tendency to increase or decrease in the concentrations of such fractions, because these variations could be indicative of the onset of an undiagnosed disease. Similarly, in pathological SPE, slight alterations in signals and concentrations should be analyzed, leading the clinician to make decisions that allow confirmatory studies to be performed for the purpose of early diagnosis. On the other hand, rigorous interpretation of the behavior of all fractions and their main ratios (albumin-γ-globulin, α1-globulin- α2-globulin and β1-globulin- β2-globulin) must be considered in the case of pathological SPE.

Among the limitations of this study is that it is a retrospective study based on the analysis of a database that is sometimes incomplete at the time of obtaining data and laboratory test results, which leads to a decrease in the sample size. On the other hand, the database used corresponds only to a single clinical laboratory in the province of Manabí; for future research, multicenter studies should be carried out in other provinces of Ecuador. This work generated an important contribution to the health sciences because it was approached from a quantitative perspective supported by classical statistical methods and data mining techniques such as factor analysis based on the extraction of principal components. This is possibly one of the first studies of dysproteinemias based on data science in Ecuador. Likewise, this work confirmed that dysproteinemias, particularly MG, occur at high frequency in the province of Manabí and seem to be increasing. It has been reported that this type of disease is rare and mainly affects older adults; however, in this work it was also found in younger people. The importance of a diagnosis based not only on visual inspection of the electrophoretogram is emphasized, because although in the non-pathological SPE the median concentration of total proteins was within the reference range, the minimum and maximum values were found to be altered. This aspect should be analyzed by the clinician because it could be the onset of an undiagnosed disease that causes slight alterations in the total protein concentration and is not yet manifested in the SPE signal pattern, which would consequently lead to a biased visual inspection. Statistically, in this work, it was demonstrated that such alterations are occurring and, if they are not addressed in time, patients may be diagnosed late.

## CONCLUSIONS

The samples were characterized by being composed mainly of patients with pathological SPE, with a predominance of female patients in the adult and elderly age groups, with the highest frequency of deaths occurring in females. The main pathologies were HyperG, followed by AI and MG, with deaths in the same order. Females were most affected by HyperG and males by AI and MG. These findings demonstrate the need for a rigorous analysis of globulin concentration in both pathological and non-pathological SPE to avoid a biased diagnosis based solely on visual inspection of the bands in the electrophoretogram. Likewise, special attention should be paid to the relationships between fractions that show the greatest influence on SPE results.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### Author Contributions

GACG - Conceptualization, investigation, statistical analysis of the database, data visualization, methodology, writing original draft, writing review & editing. YFR - Designed the

research, statistical analysis review, writing original draft. NRM - Data visualization, writing review & editing. AZC - Elaboration of the patient database. DTP - Database cleaning and parameterization of the dataset. IHA - Funding acquisition, conceptualization, investigation, writing original draft. All authors have read and approved the final manuscript.

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**Data Availability Statement**

The data that support the findings of this study are available on request from the corresponding author

**Declaration of Use of AI in Scientific Writing**

Authors declare that there was no use of generative AI and AI-assisted technologies in the writing process.

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