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Reference change values of M-protein, free light chain and immunoglobulins in monoclonal gammopathy

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ABSTRACT

Objectives: Clinical decisions in patients with monoclonal gammopathies may be highly imprecise because of variations of parameters used in diagnosis. In this study, we aimed to calculate the variation in M-protein, free light chains (FLCs), and immunoglobulins in respective patients.

Design & methods: We analyzed the data of clinically stable patients with monoclonal gammopathy (MG), which were monitored for 7-years to determine the biological variations and reference change values (RCV) of serum M-protein, monoclonal serum FLCs and immunoglobulin (Ig) concentrations. Patients that were included in the study had no change in diagnosis and showed < 5 g/L change in serum M-protein during the monitoring. From the patients included at least 3 consecutive samples were analyzed within 8 months and 7 years of initial diagnosis.

Results: The total coefficient of variations (CV) was calculated for M-protein and involved/uninvolved fractions of FLCs and immunoglobulins. From 38 patients and 456 samples that were included in the study, the total CVs were calculated for serial M-proteins (8.9%), serum involved FLCs (iFLC, 21.4%), involved Ig (i-Ig, 8.7%) and uninvolved Ig (u-Ig, 9.1%). Combining these CVs and the interassay analytical CVs, we calculated the biological CV for the serum M-protein (8.4%), serum iFLC concentration (21.1%), i-Ig (8.6%) and u-Ig (9.0%). A significant correlation was found in multiple myeloma patients between the κ/λ light chain ratio (rFLC) with i-Ig, the difference between i-Ig level and u-Ig level (d-Ig) and ratio Ig (r-Ig) (r = 0.790, 0.703 and 0.711, respectively). These correlations were not found in patients suffering from MG of undetermined significance and smoldering multiple myeloma.

Conclusions: i-Ig determinations may be an alternative to M-protein for MGs. The variations in serum FLC measurements during MG monitoring were greater than those observed in serum M-proteins and therefore need to be more rigorously revised for recommendations.

1. Introduction

Monoclonal gammopathies (MG) are characterized by the excessive propagation of plasma cells. These disorders have a unique feature, namely the secretion of immunologic and electrophoretic homogeneous monoclonal proteins (M-protein) [1]. Among the majority of MG, multiple myeloma (MM) accounts for approximately 1% of all types of malignancies in Europe [2]. In general, MM is the last stage of MG of undetermined significance (MGUS) and smoldering multiple myeloma (SMM), which are considered a clinically-defined intermediate stage [3]. Because MGUS is responsible for 50% of the detected M-protein, and MM for 35%, the measurement of M-protein becomes an important factor in these diseases [4]. In particular, the usage of M-protein as a biomarker significantly increased in determining relapses. Established guidelines recommend the diagnostic differentiation between MM, MGUS, and SMM [5]. Furthermore, FLC levels are presently used for monitoring relapse and as predictors for progression [6,7]. The re-activation of myeloma cells can produce an FLC that can be above the upper limits before alterations in M-protein are detectable. The criteria were updated in 2014 by the International Myeloma Working Group (IMWG) and serum FLC determination as well as κ/λ ratio calculation was introduced as an indicator of malignancy [8]. Recommendation of

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these parameters for patients with MG had several purposes including screening for respective disease, estimating the prognosis, deciding the therapy, and monitoring changes in the course of the disease. Moreover, FLC is recommended for the monitoring of all patients, in which the M-protein is hardly detectable or not quantifiable [9]. Since the combination of serum protein electrophoresis (SPEP), serum immunofixation (sIFE) and serum FLC quantification offers the highest sensitivity (98.6%) for detection of MGs, these three methods are broadly considered as the gold standard for MG screening [10]. According to the guidelines, the use of serum M-protein to monitor MM disease is recommended if its concentration is ≥10 g/L [11]. If the M-protein level is < 10 g/L, the sFLC level should be preferred for monitoring. However, the IMWG has recommended the use of sFLC concentrations under two conditions. One constellation is when the involved FLC (iFLC) concentration is ≥ 100 mg/L and the second is the presence of an abnormal κ/λ ratio (rFLC). As a minimal and partial response criterion, a 25% and 50% reduction in iFLC was suggested, comparable to the M-protein criteria [5]. Similarly, if the M-protein (IgG) is > 20–30 g/L, serum immunoglobulin concentrations can be used, particularly in evaluation MGs, as they often serve as quality control for changes in the M-protein [12]. Immunoglobulin levels are also diagnostically valuable when monoclonal IgA or, rarely, IgM is hidden by the β-fraction in the electrophoresis. For monitoring disease progression and response to treatment of patients with MGs, serial M-protein and FLC determination is indispensable [13]. In addition to M-protein and FLC, uninvolved immunoglobulin (u-Ig) levels have the potential to monitor the response to multiple myeloma therapy [14]. Under these perspectives, the determination of Ig variations also has become an important tool in the evaluation of these diseases.

Accurate clinical decisions in patients with MGs during disease progression monitoring and evaluation of therapy response with parameters such as M-protein, FLC or immunoglobulins relate to the variation of each parameter. In addition to the significance of the differences between laboratory results, referred to as analytical variation (CVa), there is a lot of evidence of personal variability in the occurrence and progression of the disease [15]. Analytical variation of routine quality control data can be precisely determined. However, the biological variation (CVi) in subjects and vary widely, which can make clinical interpretation difficult. In addition, there is a requirement to perform many serial measurements in stable patients with MG to collect meaningful data for individual analytes [16,17]. In respective studies, the CVi values of M-protein were determined by gel electrophoresis, but approximately 30% of the laboratories worldwide are measuring M-protein with capillary zone electrophoretic assay (CZE), excluding the United States, in which about 50% use this methodology [18]. Actually, there is no data available specifying the variations of M-protein determined with CZE, although CZE specificity is superior to gel electrophoresis [19].

This study is the first report from Europe that aims to provide calculated values for the variation in M-protein, FLCs, and immunoglobulins. The calculated values should be helpful to improve recommendations for disease monitoring.

2. Material and methods

2.1. Patients

We performed a retrospective study in which archived results from patients suffering from MG were analyzed. All data were collected by the medical care centre Labor MVZ Dr. Stein + Kollegen (Mönchengladbach, Germany), which is one of the largest providers of laboratory testing in Germany. Patients who met the IMWG criteria for MGUS, SMM and MM were included in our study. Clinically stable patients without evidence of disease progression and infection were determined from the follow-up of patients. In particular, none of the patients selected displayed features of the CRAB criteria (hypercalcemia, renal disease, anemia or bone lesions) or signs of end-organ damage. Moreover, the patients included in the study showed no change in diagnosis and had a maximum change of 5 g/L in serum M-protein during disease monitoring. Only patients having no treatment during the study interval and for which all laboratory measurements were available were included. The selection criteria of the samples were taken within 7 years and consisted of at least 3 serial samples from which all test results were available in the clinical history. Considering these criteria, a total of 45 stable patients from the database were primarily included. Finally, seven of them were excluded; for five subjects precise information about stability was missing, and two of the patients had double monoclonal bands in protein electrophoresis that may interfere with standardization of the study. The patient demographics are presented in Supplementary Table S1. We further stratified our overall cohort into three additional groups, which were MGUS, SMM, and MM. The study as presented was approved by the ethics commission located at the RWTH University Hospital Aachen (EK158/18, permission date: 26 July 2018).

2.2. Laboratory methods

Automatic CAPILLARYS 2 system (Sebia, Norcross, GA, USA) was used for detection and measurement of M-protein. To quantify the M-protein, the percentage of CZE-derived M-protein was multiplied by the total protein content, which was measured by the chemical precipitation method using reagents from Roche Diagnostics (Roche Cobas 8000 modular analyzer, Mannheim, Germany). Serum immunofixation assays were performed to evaluate γ, α, μ, κ, and λ Ig chains using the agarose gel electrophoresis technique on Hydrases 2 Sebia Hydragel 2 immunofixation on a Hydrasys instrument (Sebia). Serum FLC were determined in enhanced immunonephelometry using a BN ProSpec analyzer and N Latex FLC kit assays (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). We further performed Ig measurements on the Roche Cobas 8000 platform with immunoturbidimetric methods.

2.3. Definitions

Elevated involved immunoglobulins (i-Ig) from the different immunoglobulin classes (IgG, IgM or IgA) are defined as increased intact Ig type and monoclonal Ig (M-protein). Uninvolved Ig (u-Ig) indicates the rest of the different Ig types from M-protein and represents a value for the polyclonal immunoglobulins [20]. Simply, the difference between the total immunoglobulin levels and i-Ig values gives u-Ig. d-Ig referred to the difference between the i-Ig and u-Ig levels, which can be achieved by subtracting the sum of uninvolved immunoglobulin levels (u-Ig) from involved immunoglobulin level (i-Ig). The Ig-pair ratio (r-Ig) is defined as the ratio of involved/uninvolved Ig. Involved FLC (iFLC), FLC-pair ratio (rFLC) and the difference between involved and uninvolved free light chain (dFLC) were defined as previously described [5].

2.4. Statistical analysis

Statistical analyses were performed using the statistical package SPSS v 20.0 (SPSS Inc., Chicago, IL, USA) and Excel 2010. A p-value < .05 was considered statistically significant.

Our data were not log-transformed owing to the observed homogeneity of data distributions and goodness of fit for the linear models in our study. The biological variation, namely the within-subject biological variation (CVi) was calculated using a nested analysis of variance [21]. Briefly, the variation within an individual was defined as the standard deviation (SD) about the fitted line for each person. Therefore a linear model was selected for each parameter which was fitted to serial measurements of individual patients. To calculate the total CV, SD of the individual patient was first calculated from the error around each linear model as the mean square error using ANOVA on the

laboratory values. A single fit was done including all individuals, with separate slope and intercept parameters per person. With this procedure, we determined the total within-subject variance (CVT; including biological and analytical variances). According to the previously prescribed formula $CVT^2 = (CVa^2 + CVi^2)$, the analytical variation (CVa) was subtracted from CVT to generate CVi [17]. We calculated CVa of the methods from the interassay CVs, which were derived from the same patient samples and quality control materials used in our clinical laboratory. As quality control materials to determine the variance of immunoglobulins, FLCs, and SPEP, own trademark of the parameters such as Roche, BN II, and Sebia were used respectively. Reference change values (RCVs) were calculated from the equation: $RCV = 2^{1/2} \times Z \times (CVa^2 + CVi^2)^{1/2}$, in which Z is the probability selected for significance (a Z value of 1.96 was selected for 95% probability corresponding to a significant change). Protein fractions of SPEP were automatically identified, unless the result was inconsistent and required manual re-evaluation. For the monitoring of the analytical CV of the monoclonal protein, the sera of patients with MGUS were aliquoted and used as quality control material.

3. Results

We selected 38 patients for final evaluation with a minimum of 3 observations per patient and a maximum of 48 observations (median 9) available. All patients had monoclonal proteins of the IgG (66%), IgM (13%) or IgA (21%) isotype and received a diagnosis of MGUS (50%), SMM (11%) or MM (39%), respectively. The mean for serum M-protein concentration was 9.95 g/L (1–37 g/L). The rFLC was between 0.001 and 1766, while r-Ig was between 0.04 and 298. The M-protein levels of patients included in our study have shown both a stable and slowly increasing pattern over time. Individual patient CVs were calculated from the patient sample series and the generated graph from the distribution of CVs in relation to the median concentration of the sample series was analyzed (Fig. 1).

The total CVs for serial measurements of serum M-protein, determined FLC fractions (iFLC, uFLC, rFLC, dFLC), and the different Ig fractions (i-Ig, u-Ig, r-Ig, d-Ig) are depicted in Table 1. The CVa of the individual assays for M-protein, iFLC, uFLC, i-Ig and u-Ig as taken from the intra- and inter-assay CV of our central laboratory quality control documentation are represented in Table 2. RCV values of M-protein, u(Ig) and i(Ig) were in acceptable range (≤25%) but iFLC and uFLC were very high (> 40%) rFLC and dFLC were extremely high (> 60%) (Table 2).

In our study, the overall within-person biological variation was used here as an aggregate variable for estimating preanalytical variation, disease variation, and biological variation. Several correlations between parameters in the study group were found to be significant ($p < .001$). M-protein correlated with i-Ig ($r = 0.903$), d-Ig ($r = 0.879$) and albumin ($r = -0.715$). In addition, β-globulin correlated with IgA ($r = 0.716$) and we have also found a significant correlation in the MM

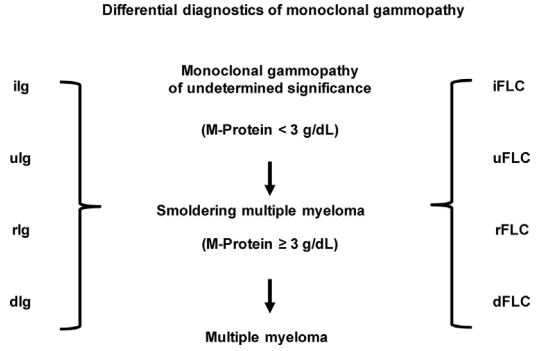

Fig. 1. %CV in individual patients relative to the median values.

Table 1
Total CVs for patient sets[a].

	MM	SMM	MGUS	Total
n	15	4	19	38
M-Protein %	9.7	5.1	9.1	8.9
i-Ig%	9.4	5.9	7.1	8.7
u-Ig%	12.5	9.3	6.7	9.1
r-Ig%	14.5	8.5	8.9	10.2
d-Ig%	11.1	7.9	9.8	9.8
iFLC%	29.6	22.1	14.1	21.4
uFLC%	21.8	12.7	15.4	16.4
rFLC%	33.1	25.4	15.3	23.6
dFLC%	45.1	35.4	21.6	31.8

[a] Abbreviations used are: dFLC, difference between involved and uninvolved free light chain; d-Ig, difference between i-Ig level and u-Ig level; iFLC, involved free light chain; i-Ig, involved immunoglobulin; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; rFLC, free light chain ratio; r-Ig, ratio between involved and uninvolved immunoglobulin; SMM; smoldering multiple myeloma; uFLC, uninvolved free light chain; u-Ig, uninvolved immunoglobulin.

Table 2
Variations of the parameters[a].

	M-protein	iFLC	uFLC	rFLC	dFLC	i-Ig	u-Ig
Total CV %	8.9	21.4	16.4	23.6	31.8	8.7	9.1
Analytical CV %	2.7	3.8	4.1	NA	NA	1.3	1.4
Biological CV %	8.4	21.1	15.9	NA	NA	8.6	9.0
RCV %	25	59	45	65	88	24	25

[a] Abbreviations used are: CV, coefficient of variation; dFLC, difference between involved and uninvolved free light chain; iFLC, involved free light chain; i-Ig, involved immunoglobulin; NA, not applicable; RCV, reference change value; rFLC, κ/λ light chain ratio; uFLC, uninvolved free light chain; u-Ig, uninvolved immunoglobulin.

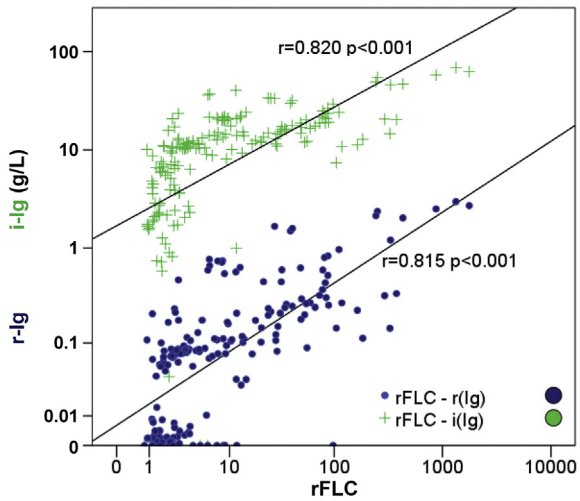

Fig. 2. Correlations between rFLC with r-Ig and i-Ig.

patients between rFLC with i-Ig ($r = 0.820$) and r-Ig ($r = 0.815$) (Fig. 2). The correlation between the same parameters in SMM patients was moderate, but not found in MGUS patients.

4. Discussion

There are only limited studies available that accurately determine the degree of change in M-protein as a measure of changes in the clinical situation. The IMWG recommended the quantitation of the monoclonal protein to monitor the progression of disease and response to treatment. In these guidelines, it is stated that a 25% or 50% decline

in serum M-protein reflects a minimal or partial response, respectively [22].

In our study, which monitored disease progression in stable patients, the total variation of M-protein was 8.9%. This variation reflects CVa (2.7%) and CVi (8.5%). These results are consistent with the study of Katzmann and colleagues, who analyzed the 5-year biological variation of M-protein in a cohort of 158 patients [16]. Contrarily, a second more recent study of M-protein variation conducted by Salamatmanesh and colleagues found higher CVi values (12.9%) in an approximately two-fold longer timeframe in a smaller cohort group ($n = 16$) of clinically stable MGUS patients [17]. The RCV values determined for M-protein by us (25%), Katzmann (22%), and Salamatmanesh (37–40%) correlated well with the CVi values. Relatively lower values in our and the previous study for M-protein variation [16] may result from the selection of patients with overall lower M-protein values (< 5 g/L), which may be considered as a major limitation, while the data reported by Salamatmanesh and coworkers was derived from clinically stable patients without exclusion criteria [17].

Katzmann and colleagues found a 50% difference between the serum M-protein CVi and i-Ig (7.8% vs. 12.3). They explained this difference with longer study intervals and intrinsic disease variation in the analyzed cohort of diseased patients [16]. In our study, the M-protein CVi was nearly the same with the CVi of both i-Ig and u-Ig (8.4% vs. 8.6% and 8.9%), presumably owing to a very strict selection of stable cases (Table 2).

Some studies confirmed that evolving changes in monoclonality of i-Ig and M-protein are useful as independent risk factors for progression to MM [20]. Correlation between M-protein and i-Ig ($r = 0.903$) also support the theory that predicts the interchangeable usage of these two parameters in the monitoring of MG patients. Our results also support u-Ig, which is used in monitoring the response to treatment, as a good parameter with a lower CVi [20]. The usage of the Ig instead of the M-protein in monitoring of MGs offers several advantages such as better test standardization, the possibility to determine the test with an automated analysis system, independency from manual handling, and factors resulting from interpretation by different operators.

There is an important role for FLC analysis in MGUS patients in the calculation of risk prediction and the probability of progression [23]. In line, some studies suggest that serum should be used instead of 24-h urine for assessing monoclonal FLC as a measure for response in MM patients [24]. A recent study highlighted the relationship between high serum FLC levels in diagnosis and severity of renal failure in MM [25]. rFLC is also used as a sensitive marker of clonality. Excessive clonal production of only one type of FLC results in an increased rFLC ratio in patients with MGs [26]. Obviously, the use of rFLC in diagnosis is useful for compensating for changes in patients with reduced renal function.

The correlation in this study between rFLC and involved Ig was $r = 0.815$ ($p < .001$) and between rFLC and ratio Ig was $r = 0.820$ ($p < .001$) in MM patients supporting the important role of rFLC in diagnostics. The iFLC is an important parameter for monitoring oligosecretory MM [5], whereas for primary amyloid the dFLC has been recommended to normalize the effects of declining renal clearance [27]. In our study, CVi of the serum iFLC was 21.1%, which is consistent with the previous study on iFLC variation (28.4%) performed by Katzmann and colleagues [16]. These higher CVi values of the iFLC serum resulted in higher RCV values in both studies (59.3% and 78.7%, respectively).

It should be noted that the method for FLC determination used in previous studies was different (Freelite) than ours (N-latex FLC assay). However, the RCV% values that we have calculated as ratios should correct for potential interferences resulting from different test methods. FLCs have low molecular weight and short serum half-life compared to intact immunoglobulins. FLC concentrations are also very rapidly affected by changes in plasma cell synthesis and renal clearance. Due to physiological and molecular features, FLCs may have larger CVs than M-protein [16]. The IMWG guidelines predict a very good partial response of 90% in reduction of dFLC, and a partial response of at least 50% reduction in dFLC [13]. However, we observed a RCV of 88% for dFLC. This means that a 50% reduction in dFLC may be due to analytical or biological variations. Furthermore, the IMWG recommended normalization of rFLC for a stringent complete response [13]. In our study, we found a 65% RCV for rFLC suggesting that the normal limits of rFLC should be given with confidence intervals, because otherwise, border values may be interpreted falsely.

In addition to the intrinsic properties of monoclonal proteins, differences in the performance between assays, variability resulting from the usage of non-standard reagents and different analysis platforms can also contribute to significant variations in test results. Therefore, it is important to understand the utility and limitations of each factor [28]. From the perspective of various platforms for M-protein, the CZE results with SPEP showed approximately the same CV values compared to previous studies [16].

In the MM group, we found a positive correlation between rFLC with i-Ig and r-Ig (Fig. 2), but the correlation in SMM patients was only moderate, and not found at all in MGUS patients.

In summary, our data confirm the guidelines proposed to standardize treatment-response criteria using serum M-protein and Ig levels. However, we suggest revising the respective diagnostic guidelines.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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