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Published in:
Clinical Chemistry and Laboratory Medicine

DOI:
10.1515/cclm-2015-0792

Publication date:
2016

Document version:
Final published version

Citation for pulished version (APA):
Hansen, C. T. (2016). Performance goals for immunoglobulins and serum free light chain measurements in plasma cell dyscrasias can be based on biological variation. *Clinical Chemistry and Laboratory Medicine*, *54*(6), 1031–1033. https://doi.org/10.1515/cclm-2015-0792

Mini Review

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Performance goals for immunoglobulins and serum free light chain measurements in plasma cell dyscrasias can be based on biological variation

DOI 10.1515/cclm-2015-0792
Received August 17, 2015; accepted January 2, 2016; previously published online January 29, 2016

Abstract: Measurements of immunoglobulins and serum free light chains (sFLC) are frequently used in patients with monoclonal plasma cell dyscrasia (PCD). For optimum patient care, well-defined performance standards or goals for the measured concentrations of immunoglobulins and sFLC are required. Generally, data based on biological variation is a good and reliable method for setting desirable performance standards; this also applies for the measurements of paraprotein and sFLC. The benefits of this approach are several. Among others, it is independent of the clinician, and it provides us with information about reference change value and index of individuality. Several studies on biological variation of both immunoglobulins and sFLC have been published, and mostly the studies are well performed. The studies normally show small within-subject biological variation resulting in strict analytical goals, which in most cases are difficult to meet. Nevertheless, we still need further information on biological variation of immunoglobulins and sFLC in patients with PCD and in the elderly, which are the main target populations for the two measurands. Furthermore, to improve data on biological variation of immunoglobulins and sFLC, studies accounting for number of individuals, samples, and replicates, as well as time length of the studies are needed.

Keywords: biological variation; free light chains; paraprotein; performance standards.

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Introduction

Measurements of immunoglobulins and free light chains in serum are frequently used when diagnosing and monitoring patients with monoclonal plasma cell dyscrasia (PCD), primarily multiple myeloma (MM), AL amyloidosis and monoclonal gammopathy of undetermined significance (MGUS) [1]. Additionally, baseline measurements of serum free light chains (sFLC) are of major prognostic value in virtually every PCD, with an abnormal ratio between free κ chains to free λ light chains (rFLC) being an adverse prognostic marker. Finally, according to the international response criteria, measurements of both monoclonal protein (M-protein) and sFLC are needed for the response evaluation of MM and AL amyloidosis patients after cytoreductive treatment [2].

For clinicians to be able to rely on the measured concentrations of the immunoglobulins and sFLC, desirable test performances, especially imprecision and bias, need to be well defined to fulfill medical needs. For over 50 years, the setting of analytical quality specifications in laboratory medicine has been a matter of discussion and debate. Objective standards should always be used in laboratory practice to facilitate the provision of optimum patient care. In 1999 at the Stockholm Consensus Conference on quality specifications in laboratory medicine the initial proposal for the levels of approach for setting performance standards was made [3]. According to the latest consensus statement concerning analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine analytical performance goals should be based on a) clinical outcomes, b) components of biological variation, or c) state of the art. Depending on the measurand, some of the above are more suited than others to defining performance goals [4].

A good and reliable, though circuitous method for setting desirable performance standards is using data based on components of biological variation, using the following formulas:

$$CV_A\% \leq 0.5 * CV_I$$

$$\text{Bias} < 0.25 * \sqrt{(CV_I^2 + CV_G^2)}$$

$$\text{Total error} < 1.65 * CV_A + \text{Bias}, \; p < 0.05$$

CV_A=analytical variation; CV_I=within-subject biological variation; CV_G=between-subject biological variation.

The advantages to this approach, in the context of measurements of immunoglobulins and sFLC testing are several. Firstly, it is independent of the clinician's sense of a significant medical change, which is often unreliable and shows that clinicians may have a poor understanding of sources of test-result variation. Secondly, it minimizes the ratio of 'analytical noise' to the biological signal. Thirdly, it can be applied to most measurands for which population-based or subject-specific biological variation data can be established. Fourthly, data on biological variation provides us with more information than just being able to set analytical goals; it provides us with the possibility of calculating reference change value (RCV), the difference needed between two measurements in serial monitoring to be significantly different, and further allows the calculation of the index of individuality (CV_I/CV_G), which gives us valuable information about the usefulness of a normal reference interval in the diagnostic setting. Limitations of the method are first of all that studies on biological variation are consuming to perform. Also, care must be exercised in assessing the relevance and validity of the data [5]. Another problem with the use of data on biological variation is the fact that the derived performance standards can be very stringent, because homeostatic control mechanisms are finely adjusted. Current technology does not always allow these performance goals to be achieved. They should, however, be viewed as targets worthy of achievement, for both manufacturers and clinical chemists.

There is published data on biological variation for both immunoglobulins and sFLC.

Normal immunoglobulins

The biological variation for immunoglobulins has been studied many years ago [6–8]. The obtained CV_I and CV_G in the studies are very similar, with only small deviations, and minimal impact on the desirable performance standards. Generally, CV_I is low, the average values are: IgG 4.0%, IgA 4.6%, and IgM 5.0%. CV_G is somewhat higher, the average values are: IgG 17.4%, IgA 44.8%, and IgM 49.9%. The consequence of the small CV_I is that the desirable performance goals will be very stringent. In most cases the analytical goals are difficult to meet, especially when using reagents with different lot numbers. In the studies, however, CV_A attained the analytical goals. The limitations of the studies are that only relatively young (23–48 years), ostensibly healthy individuals were investigated. A limited number of individuals were tested [9–11] with a balanced ratio between men and women. The time length of the studies varies from 1 week, over 4 weeks to 3–4 months, with 6–10 samples from each subject. The performed studies used the analytical technique that is the current practice in laboratory testing to day, which is necessary for correct interpretation of data. What is not taken into account in these studies is the fact that biological variation of immunoglobulins can be altered in patients with PCD. Studies looking at this aspect could improve the use of the derived data, providing both the desirable performance standards, and knowledge on the RCV.

Normal serum free light chains

The performed studies on biological variation for sFLC measurements are more recent [12, 13]. CV_I of sFLC is generally small compared to the reference interval, at 4.3% and 8.1% for κFLC, and 7.6% and 7.0% for λFLC, in the two respective studies. The small CV_I results in strict analytical goals, which can be difficult to attain due to limitations in current technology, and the use of an analysis based on polyclonal antibodies. CV_G for κFLC is 21.0% and 14.1% and for λFLC 30.0% and 27.5%, and thus, considerably higher than CV_I. The number of individuals studied was 18 and 21, and more females than males were tested. Again, the individuals were young (23–54 years) and healthy, though Hansen et al. studied the biological variation in patients with PCD, namely MGUS and MM. They found comparable CV_I between healthy individuals and patients with PCD, thus, strengthening the use of RCV, and thereby improving the clinical monitoring of patients with PCD [12]. The sampling period of the studies varied from only 5 days to over 2 months to 3–4 months, with five to 10 samples taken from each subject. The studies were performed using the analytical assay from FreeLite® (The Binding Site Group Ltd, Birmingham, UK), which presently is the most frequently used assay for analyzing sFLC. The studies were performed on two different platforms. Katzmann et al. have assessed the biological variation of sFLC in patients with PCD, and involved FLC over 100 mg/L, and found a very high CV_I of 27.8%, however CV_I was determined over a 5-year period and therefore not surprisingly large. Further,

no information about homogeneity of the results was presented in the paper, making the result less useful for setting performance standards or RCV [14].

Generally, the studies of biological variation of both immunoglobulins and sFLC are well performed, though, only partly fulfilling the new proposed checklist for critical appraisal of studies of biological variation [9].

To optimize the use of data on biological variation in the setting of desirable performance standards we need further information on biological variation in patients with PCD and in the elderly, which are the main target populations for the two measurands. Also, we need to consider the number of individuals studied, as well as the time length of the studies. Fraser and Harris stated that "The components of variation can be obtained from a relatively small number of subjects over a reasonably short period of time" [10], but this statement stands unexplained and scientifically unsubstantiated. Røraas et al. have looked into this issue, and find that the results obtained in studies on biological variation depend on the ratio between CV_A and CV_I. A small CV_A is vital to gain sufficient power in the experiment. In most situations they find that there is greater benefit from increasing the number of samples from each individual instead of increasing the number of individuals. Also there is higher gain by increasing the number of samples rather than increasing the number of replicates [11]. The ratio between CV_A and CV_I is small for both immunoglobulins and sFLC, and according to the calculations of Røraas et al. a powerful study-design for assessing biological variation of the two measurands will be 20 individuals having taken 10 samples with two replicates, which will provide a power of 1.0, and an acceptable confidence interval of 33%. When assessing the biological variation in patients with PCD a proper time length of the study will be 3–4 weeks, which in my opinion will be long enough to investigate the homeostatic set point, and not too long, which would risk slight disease progression and biasing the results.

In conclusion, setting desirable performance goals using data of biological variation is reliable, bearing in mind, that when CV_I is small, the standards can be hard to fulfill, but nevertheless are worth striving for. Further, we need reliable data on the biological variation for both immunoglobulins and sFLC. New studies meeting the above mentioned criteria would be welcomed and necessary if biological variation is to be the optimal approach for setting desirable performance goals.

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