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Comparison of Two Immunoassays for Serum Thyroglobulin

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ABSTRACT

Introduction: Monitoring serum Thyroglobulin (Tg) is recommended as a tumor marker in the postoperative phase of patients with differentiated thyroid cancer (DTC). Results obtained by different methods may vary causing erroneous interpretations of the results during the follow-up. Also circulating anti-thyroglobulin antibodies (TgAb) may interfere differently depending on the method. This study compares the levels of serum Tg quantified by two different diagnostic platforms, both based on the ICMA method, to evaluate possible erroneous interpretations that could occur during the transition to a new instrument. **Materials and Methods:** 60 serum samples, randomly selected from the hospital daily routine, were tested through the newly Tg assay (ArchitectAbbott) and the currently used Tg assay (Medical System). On 51of 60 samples anti-Tg antibodies were also tested. Statistical elaboration was performed by Passing-Bablok and Bland Altman analyses (MedCalc Statistical Software version 19.1.3). **Results:** The regression line according to Passing-Bablok is y=-0,130+0,769x; the Intercept 95% CI was -0,4635 to 0,1454; the Slope 95%CI was 0,7321 to 0,8053. Cusumtest (p= 0,56). Cohen's coefficient K=0.78 (95% CI 0.49 – 1.00). Bland-Altman analysis show a bias mean of 3,7 (95% CI 1,8043 to 5,5870), Lower limit -10,6543 and Upper Limit 18,0456. **Conclusion:** Results comparison showed a moderate agreement between the two instruments. A proportional bias indicates a systematic underestimation of 28% for Architect compared to Immulite, and these differences are not influenced by the presence of TgAb. Although the difference is not statistically significant, alerting the clinician is essential to prevent misinterpretation of results, particularly during follow-up.

INTRODUCTION

Thyroglobulin (Tg) is a homodimeric glycoprotein synthesized by the epithelial cells of the thyroid gland and stored as a colloid within the follicular lumen. In the follicular cell, Tg is iodinated and degraded to thyroxin (T4) and triiodothyronine (T3) through a process regulated by thyroid-stimulating hormone (TSH). Considering that only a small amount of Tg bypasses intrathyroidal proteolysis and reaches the bloodstream in intact form, serum levels are normally low; they are related to the overall volume of thyroid tissue (1 ng/mL per 1 g of thyroid mass) and also depend on the intake of iodine and the gender of the patient. Under normal conditions, the average circulating values are between 20-25 ng/mL.

Serum Thyroglobulin is recommended as a postoperative tumor marker in the long-term follow-up of patients with differentiated thyroid cancer (DTC), the most frequent thyroid neoplasm (2).

In these patients, usually undergo a total thyroidectomy followed by radioiodine therapy, undetectable serum Tg levels are expected. An increase in the serum Tg concentration over time is suggestive of persistence or recrudescence of the tumor form (3,4). Furthermore, serum Tg level, although does not play a role in routine thyroid diagnostics, plays an important role in the differential diagnosis between hyperthyroidism and thyrotoxicosis factitia, in congenital hypothyroidism, for the differential diagnosis between agenesis and thyroid ectopia and detection of the rare forms of Tg deficiency (5,6). Currently, the most widespread analytical techniques for the thyroglobulin levels determination are the immunometric assays (IMA), in particular, the immuno-chemiluminometric assays (ICMA) due to their high sensitivity and reduced sample processing time. The most relevant analytical interference for immunometric assay for Tg is represented by the presence of Tg antibodies (TgAbs) that compromise the measurement, leading to underestimating the concentration of this glycoprotein (7,8,9).

Another confounding factor in measuring Tg levels, during a long-term follow-up, maybe due to the use of different diagnostic platforms. Indeed, even if they are based on the same method (e.g. ICMA), the results could be slightly different and lead to wrong clinical interpretations.

The purpose of this study was to compare the serum values of thyroglobulin obtained by using two Tg automated ICMA platforms available in our laboratory. Furthermore, the correlation between the results obtained and the presence of TgAbhas been evaluated.

MATERIALS AND METHODS:

The study was conducted at the Hospital of Bracciano (Rome) between August to September 2019.

60 left-over serum samples, completely anonymized, were used. Specimens were randomly selected, without considering any inclusion or exclusion criteria, and by following the guidelines of the Helsinki Declaration on human experimentation. Two aliquots of approximately 200µl of each serum were prepared within two hours of centrifugation. Tg and TgAbs concentrations were measured with the new automated immunochemiluminometricassay, product for Architect i2000sr (Abbott Laboratories, Abbott Park, IL, USA). The test is based on a quantitative immunoassay in chemiluminescence at a delayed step.

The same samples were processed on the currently used Tg assay product for Immulite 2000 (Medical System, Genova, Italy). The test is based on a one-step chemiluminescence immunoassay.

The normal reference values provided by the manufacturer for the Tg are respectively between 0 and 55 IU / mL for Immulite 2000 and between 0 and 64.15 IU / mL for Architect; serum TgAb levels>4.11 IU/mL were reported as positive.

Before testing the samples and controls for Thyroglobulin were performed on both instruments. The serum aliquots were tested in duplicate and the agreement between the methods was performed using the average values obtained in the two analyzers.

TgAbs concentrations were obtained on a sub-sample of 51 sera, randomly selected from the total sample (n=60).

Statistical analysis

All statistical analyses were done using MedCalc Statistical Software version 19.1.3 (MedCalc Software, Ostend, Belgium). Data distribution was assessed through the Shapiro-Wilk test for normal distribution. The comparison of the values was analyzed through the Passing-Bablok regression analysis (95% CI), which also provided the results of the Cusum test for linearity (10,11). Cohen's kappa coefficient (κ) (95% CI) was calculated to evaluate the inter-rater agreement and the value obtained has been interpreted according to the reference intervals (12). The difference between the two methods has been described through the Bland Altman plot.

Agreement between the interpretations of the results provided by the two diagnostic platforms, were also graphically highlight by tracing the straight lines corresponding to the respective cut-off values in the Passing Bablok regression line chart (y= 55 cut-off Immulite; X= 64 cut-off Architect).

To evaluate the possible dependence among the differences of Tg concentrations found between the two diagnostic platforms (expressed as ratio% Tg Architect/TgImmulite) and the presence of TgAbs, regression analysis was used and the data were graphically represented through a scatter diagram.

RESULTS AND DISCUSSION:

RESULTS

Tg results obtained through Architect were between 0.0 and 291; the median value was 9,3 (95% CI: 6,7072 to 12,2149), Q1 and Q3 were respectively 4,6450 (95% CI : 2,0504 to 6,5679) and 18,4656 (95% CI: 12,2679 to 23,7331). Tg results provided by Immulite were between 0.2 and 300; the median value was 11,0050 (95% CI : 8,1603 to 15,5122), Q1 and Q3 were respectively 5,6450 (95% CI: 1,8897 to 8,0541) and 25,9850 (95% CI : 15,6448 to 30,4734) .

Tg Ab results provided by Architect were between 0.2 and 1000; the median value was 1,0550 (95% CI : 0,7474 to 2,3207), Q1 and Q3 were respectively 0,6150 (95% CI : 0,4871 to 0,7558) and 3,3900 (95% CI : 1,8275 to 21,7719) .

The regression line according to Passing and Bablok was y=-0,130+0,769x; the Intercept 95% CI was - 0,4635 to 0,1454; the slope was 0,7321 to 0,8053 (Figure 1).

The Cusum test for linearity has provided a P-value of 0,56. Cohen's coefficient was 0.78 (CI 95% 0.49 – 1.00).

As showed in the Bland Altman plots in figures 3, the arithmetic mean of the absolute differences between the values obtained with the two instruments, was 3,6957 (95% CI : 1,8043 to 5,5870), Lower Limit -10,6543 (95% CI -13,9046 to -7,4040), Upper Limit 18,0456 (95% CI 14,7953 to 21,2959); the arithmetic mean of percentage differences was 27,8057 (95% CI: 16,0842 to 39,5272), Lower Limit -61,1286 (95% CI -81,2724 to -40,9848), Upper Limit 116,7400 (95% CI 96,5962 to 136,8838).

Regression analysis between ratio% Tg architect/ TgImmulite and TgAb concentration has been reported in figure 4; regression line was y = 79,6126 + -0,01082 x; the Intercept 95% CI 69,6969 to 89,5333; the Slope was 0,05039 to 0,0287.

DISCUSSION:

In this study, we compared the Tg values obtained on different analytical platforms, by using commercial kits based on the same method (ICMA).

The statistical analysis carried out indicates that the results obtained are in moderate agreement and, despite the differences are not statistically significant, a systematic bias highlights an underestimation of Tg concentration by about 28%, in Architect compared to Immulite.

Moreover, as shown in figure 2, the Passing-Bablock correlation line crosses an area of the graph where the interpretations of the results obtained with the two diagnostic platforms are discordant.

This area of disagreement is delimited on the x-axis by the results obtained by Immulite ranging between 55IU / mL and 83 / IU / mL. The samples that lie in that area of the plot, interpretable as out-of-range for Immulite, would be considered as normal for Architect as they are below its reference threshold value (64.15 IU / mL on the y axis).

As already described by several authors, TgAbs interference remains a serious problem that compromises the clinical utility of Tg tests for about 20% of DTC patients who have TgAb in circulation (13,14).

This study only considered the interference of TgAb on the bias obtained between the two platforms. The differences observed between the two series of Tg values analyzed concerning the concentration of TgAbs did not reveal differences between the two instruments in terms of interference. Therefore, a possible interference of the TgAbs, if present, would act proportionally on both instruments.

Small variations of the Tg level in patients following post-thyroidectomy must be carefully interpreted and closely validated for the clinical classification of the patients with possible recurrence; any change in Tg assay has the potential to disrupt serial monitoring and prompt inappropriate clinical decisions. Assuming this, our results underline the importance for the patient to perform the monitoring of the Tg always in the same laboratory and possibly on the same diagnostic platform. If a change of platform is necessary, it is recommended to check the comparability of the results obtained through parallel Tg measurements using both the old and the new instruments. The study also suggests that endocrinologists and oncologists should be aware of changes in the platform to properly manage laboratory data.

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Figures

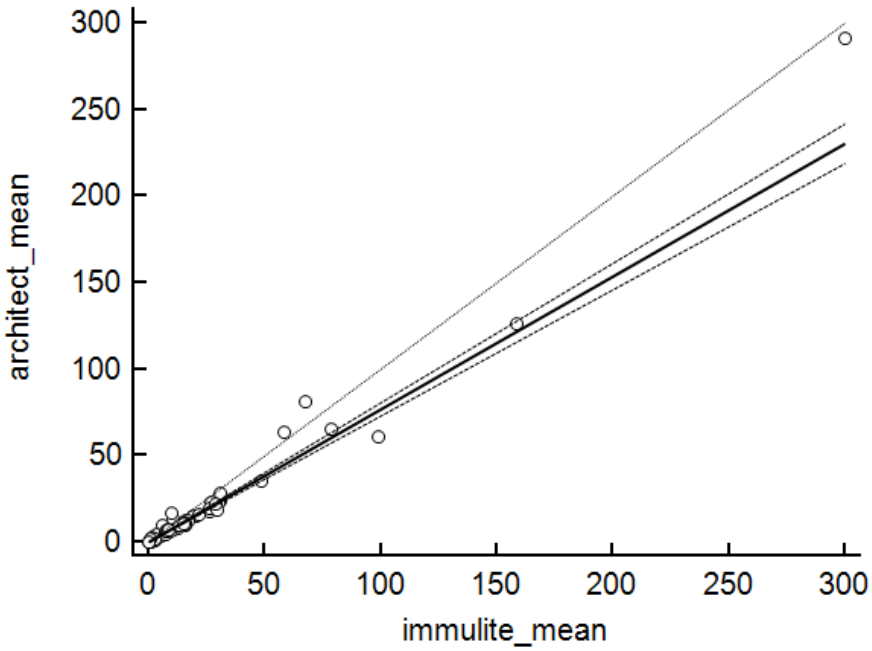

Figure No. 1: Passing-Bablok regression analysis for Tg Immulite *vs* Architect. On the x-axis the mean values of each duplicate obtained by Immulite; on the y-axis the mean values of each duplicate obtained by Architect. Regression line y=-0,130+0,769x; 95% CI -0,4635 to 0,1454 (Intercept) and 0,7321 to 0,8053 (Slope). Cusum test for linearity P=56. Sample size N=60; regression line (solid line), confidence interval for the regression line (dashed lines), identity line (x=y, dotted line)

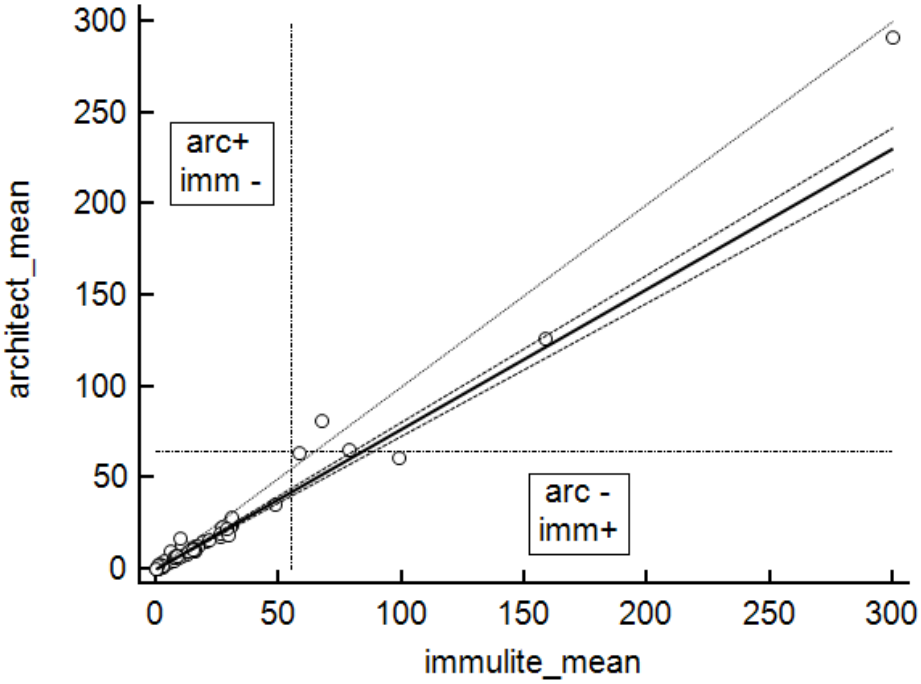

Figure No. 2: Passing Bablok plot. Areas of discordance (**arc+ imm-** and **arc- imm+**) obtained including Architect (horizontal dotted line) and Immulite (vertical dotted line) cut-off values in the graph of Passing Bablok regression line (y=64,15 and x=55) . The regression line crosses the area of the graph (**arc- imm +**) where the analyzed samples are out of range for immulite and normal in architect.

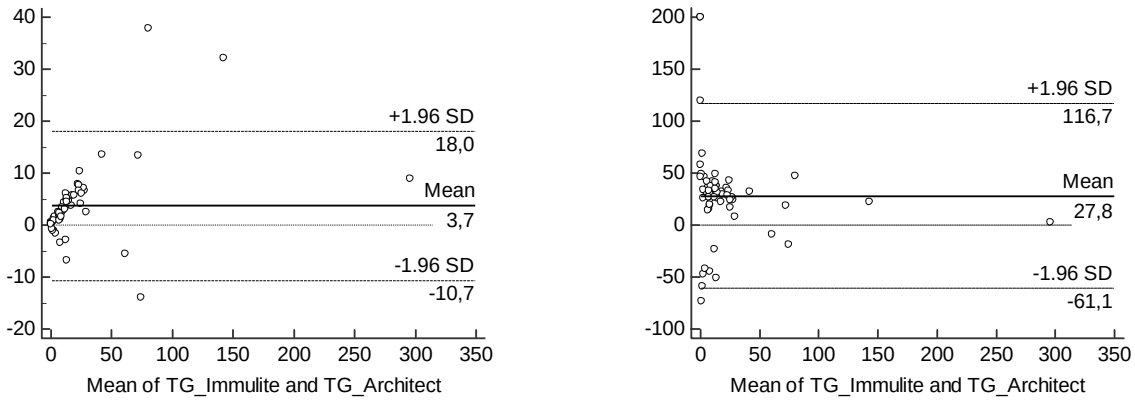

Figure No. 3: Bland-Altman plot. On the leftthe absolute differences of the Tg values between Immulite and Architect (y-axis) are plotted against the averages of the value obtained by the two diagnostic platforms (x-axis). On the right the differences of the Tg values between Immulite and Architect are expressed as %. The limits of agreement (LoA) are defined as the mean difference ± 1.96 SD of differences (longdashed line); mean difference between the two platforms (solid line); zero line (dashed line).

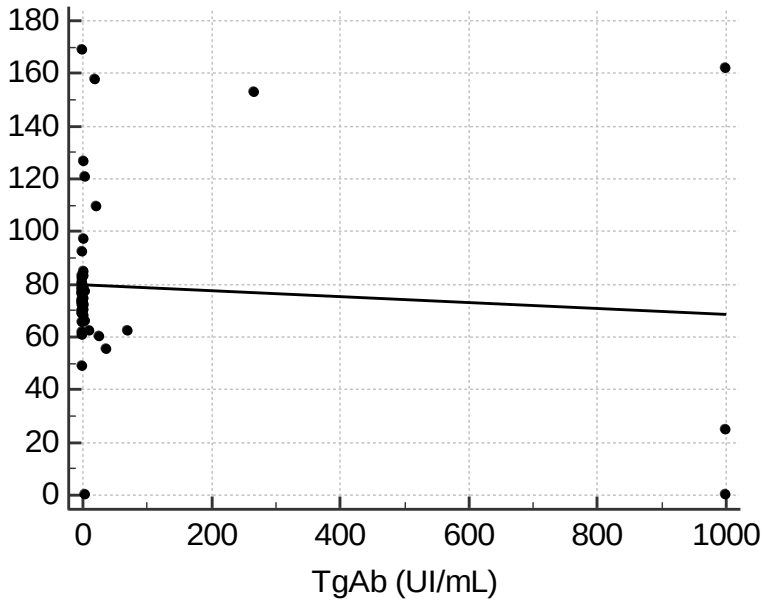

Figure No. 4: Ratio % Tg Architect/ Tg Immulite (y-axis) plotted against Tg Ab concentration (x-axis). Regression line: y = 79,6126-0,01082 x ; 95% CI 69,6969 to 89,5333 (Intercept); (Slope)-0,05039 to 0,0287.