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ORIGINAL ARTICLE

Procalcitonin Chemiluminescent Immunoassay - Bias Estimation on Multiplatform

Yun Li [1], Fengyue Lu [1], Qide Liang [1], Pingfeng Feng [2], Xin Li [2], Chaohui Duan [3], Ping Guan [1], Huayi Huang [4]

[1] *Department of Laboratory Medicine, Guangzhou Thoracic Hospital, Guangzhou, Guangdong, China*
[2] *Department of Laboratory Medicine, Nanfang Hospital of Southern Medical University, Guangzhou, Guangdong, China*
[3] *Department of Laboratory Medicine, Sun Yat-sen Memorial Hospital Affiliated to Sun Yat-sen University, Guangzhou, Guangdong, China*
[4] *Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, New York, USA*

SUMMARY

Background: **Procalcitonin (PCT) is a useful biomarker for infection and especially useful for sepsis management. Multiple platforms, including the chemiluminescence immunoassay (CLIA), have been used for serum PCT analysis. However, the results from different analytical platforms should be evaluated to determine if they can be mutually substituted in the same laboratory.**
Methods: **The serum PCT were analyzed on the Mindray CL-6000i chemiluminescent immunoassay (candidate method), the Roche Elecsys, and the VIDAS PCT chemiluminescence immunoassay platforms (comparative measurements), and the results were evaluated and compared, following the CLSI EP09-A3 guidelines, by using patient samples with different PCT concentrations.**
Results: **The median of difference was 0.04 (95% CI: 0.038 - 0.045) between the candidate method and the comparative measurements for the concentration interval of < 0.5 ng/mL. The median of percentage difference was 6.6% (95% CI: 5.5% - 8.7%) for the concentration interval of 0.5 - 2.0 ng/mL, the median of difference was 0.11 (95% CI: 0.06 - 0.17) for the concentration interval of 2.0 - 10.0 ng/mL, and the median of percentage difference was -4.7% (95% CI: -6.1% - 2.4%) for the concentration interval of 10.0 - 100.0 ng/mL. The acceptable bias was ± 0.055 (± 10.4%) at 0.53 ng/mL (low value), and the acceptable bias was ± 0.83 (± 9.0%) at 9.34 ng/mL (high value). The bias between the candidate method and comparative measurements was acceptable for the full concentration ranges.**
Conclusions: **The bias between the PCT results from Mindray, Roche, and VIDAS was acceptable. Therefore, the results of the three analytical platforms were comparable, and they may be mixed-used in the same institution.**
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Correspondence:
Ping Guan
Department of Laboratory Medicine
Guangzhou Thoracic Hospital
Guangzhou, 510095
Guangdong
China
Email: 13570935838@163.com

Huayi Huang
Department of Surgical Oncology
Roswell Park Comprehensive Cancer Center
Buffalo, New York 14263
USA
Email: Huayi.Huang@Roswellpark.org

KEYWORDS

procalcitonin (PCT), chemiluminescent immunoassay (CLIA), analytical performance, consistency, evaluation

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INTRODUCTION

Procalcitonin (PCT) is a 13-kDa peptide precursor of calcitonin consisting of 116 amino acids that was discovered more than two decades ago as a prohormone of calcitonin produced by C-cells of the thyroid gland [1,2]. In healthy subjects, the circulating levels of PCT are very low and usually undetectable [2]. After its discovery, elevated serum PCT levels were found in patients with various infections and inflammations such as lower respiratory tract infection, community-acquired pneumonia, knee arthritis, and the bacterial coinfection with COVID-19 [3-8], making it a useful biomarker for the diagnosis of infection and inflammation. In addition, PCT is used together with other biomarkers for sepsis diagnosis or prediction [9,10] as well as to guide antibiotic stewardship, although the latter is still controversial [11,12]. Various analytical platforms are available for PCT detection nowadays; one type of assay principle is the chemiluminescent immunoassay (CLIA). Different detection platforms and different analytical methods may produce inconsistent results [13] and require a comparison or evaluation [14]. Comparison is often used in the evaluation of assay consistency, which includes accuracy and bias (trueness). In this study, we evaluated the PCT measurement bias among Mindray CL-6000i (Mindray, Shenzhen, Guangdong, China), Elecsys BRAHMS PCT assay (Roche, Rotkreuz, Switzerland) on a Cobas e801, and VIDAS B.R.A.H.M.S. PCT (Biomerieux, Marcy L'Etoile, France) chemiluminescence immunoassay platforms by following the CLSI EP09-A3 [15] guidelines and by using patient samples with different PCT levels.

MATERIALS AND METHODS

Project design

This project was designed as a prospective multicenter comparison study. The comparison was carried out using patient serum samples. The samples included four different concentration intervals of PCT: < 0.5 ng/mL, 0.5 - 2.0 ng/mL, 2.0 - 10.0 ng/mL, and 10.0 - 100.0 ng/mL, regardless of whether the patient was outpatient or hospitalized.

Participating hospitals

Three class-A tertiary hospitals in Southern China, namely Guangzhou Thoracic Hospital, Nanfang Hospital of Southern Medical University, and Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University, participated in this study.

Ethical approval

This study was approved by the Institutional Review Board/Ethics Committee of Guangzhou Thoracic Hospital, Nanfang Hospital of Southern Medical University, and Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University (2020 (No. 63), NFEC-2020-235, and SKSE C-KY-KS-2022-149, respectively).

Chemiluminescence immunoassay of procalcitonin

Venous blood was drawn upon hospitalization or at the outpatient clinic visit. The samples were delivered to the clinical laboratory within 30 minutes of sampling and processed following the laboratory standard operating procedures. Serum was isolated and PCT was analyzed on the Mindray CL-6000i (Mindray, Shenzhen, Guangdong, China) using the procalcitonin (CLIA) reagent kit, on the Roche e801 (Roche Diagnostics, Rotkreuz, Switzerland) using the Elecsys BRAHMS PCT reagent kit, and on the VIDAS B.R.A.H.M.S PCT (Biomerieux, Marcy L'Etoile, France) chemiluminescence immunoassay platforms following the manufacturers' instruction. Hemolytic, lipemia, and icterus samples were excluded from the analysis.

Statistical analysis

All data were analyzed following the guidelines of CLSI EP09-A3. Data analysis was performed by a third-party statistician. The original data was stored and is available upon request.

RESULTS

Sample information

Table S1 shows the distribution of PCT samples from three different study sites with different PCT concentrations.

Distribution of difference and percentage difference of PCT detection results between candidate method and comparative measurements

Table S2 and Figure S1 show that the difference and percentage difference of data in this study are non-normal distribution in which the Kurtosis is too high with larger skewness.

Scatter plot of candidate measurements vs. comparative measurements

Figure 1A shows that the scatter of plots gets wider as the PCT detection value increases. Figure 1B indicates that the difference appears to be increasing in proportion to the concentration. There is an extreme value of -58.03 at the lower right, which is more than 3 times the interquartile range.

Figure 1C shows that the percentage difference seems to decrease proportionally to the concentration.

Difference plot of serum PCT at different concentration levels between candidate method and comparative measurements

The formula for this calculation is: difference (ng/mL) = Mindray - Merieux or Roche;

difference (%) = 100 × (Mindray - Merieux or Roche)/ Merieux or Roche

Table 1. Analytical results of difference and difference percentage (%) of PCT at < 0.5 ng/mL (n = 519).

Index	Statistic		Bootstrap (based on 1,000 Bootstrap samples)			
			bias *	standard error	95% CI	
					lower limit	upper limit
Difference (ng/mL)	mean	0.0529	0.0000	0.0032	0.0471	0.0592
	median	0.0400	0.0006	0.0015	0.0380	0.0450
Difference %	mean	30.9712	-0.0511	3.0883	25.8145	37.8044
	median	19.7772	0.2002	0.8391	18.2796	21.7386

*** The bias depicts the mean of 1,000 Bootstrap samples and its associated statistical difference.**

Table 2. Frequencies module analytical results of PCT of difference and difference percentage (%) of PCT at 0.5 - 2 ng/mL (n = 345).

Index	Statistic		Bootstrap (based on 1,000 Bootstrap samples)			
			bias *	standard error	95% CI	
					lower limit	upper limit
Difference (ng/mL)	mean	0.0799	-0.0003	0.0135	0.0541	0.1062
	median	0.0690	-0.0025	0.0081	0.0510	0.0800
Difference %	mean	8.2530	-0.0182	1.1147	6.1642	10.4737
	median	6.6236	0.2207	0.8412	5.5249	8.7248

*** The bias depicts the mean of 1,000 Bootstrap samples and its associated statistical difference.**

Table 3. Frequencies module analytical results of PCT of difference and difference percentage (%) of PCT at 2 - 10 ng/mL (n = 328).

Index	Statistic		Bootstrap (based on 1,000 Bootstrap samples)			
			bias *	standard error	95% CI	
					lower limit	upper limit
Difference (ng/mL)	mean	0.0977	-0.0007	0.0457	0.0085	0.1862
	median	0.1100	0.0037	0.0295	0.0600	0.1700
Difference %	mean	2.4787	-0.0210	1.0091	0.5160	4.4835
	median	2.2801	0.2216	0.6659	1.4753	4.2290

*** The bias depicts the mean of 1,000 Bootstrap samples and its associated statistical difference.**

Figure 2A shows a constant difference variability (constant standard deviation) under 0.5 ng/mL of comparative concentration interval. Figure 2B shows a consistent spread of the percentage differences (constant coefficient of variation) across 0.5 - 2.0 ng/mL of the comparative concentration interval.

Since the data points in Figure 2A basically conform to the band distribution, the difference was analyzed using the frequencies module of SPSS, with results shown in Table 1.

Data points in Figure 2B basically conform to the band distribution, and the percentage difference was analyzed using the frequencies module of SPSS, with results shown in Table 2.

Figure 2C shows a consistent spread of the differences (constant standard deviation) across 2.0 - 10.0 ng/mL of

Table 4. Frequencies module analytical results of PCT of difference and difference percentage (%) of PCT at 10 - 100 ng/mL (n = 133).

Index	Statistic		Bootstrap (based on 1,000 Bootstrap samples)			
			bias *	standard error	95% CI	
					lower limit	upper limit
Difference (ng/mL)	mean	-1.9639	0.0302	0.6572	-3.3061	-0.7001
	median	-0.6300	-0.1061	0.2273	-1.2300	-0.3800
Difference %	mean	-4.2220	0.0736	1.5025	-7.1826	-1.1903
	median	-4.6888	0.3219	0.9985	-6.0543	-2.4141

*** The bias depicts the mean of 1,000 Bootstrap samples and its associated statistical difference.**

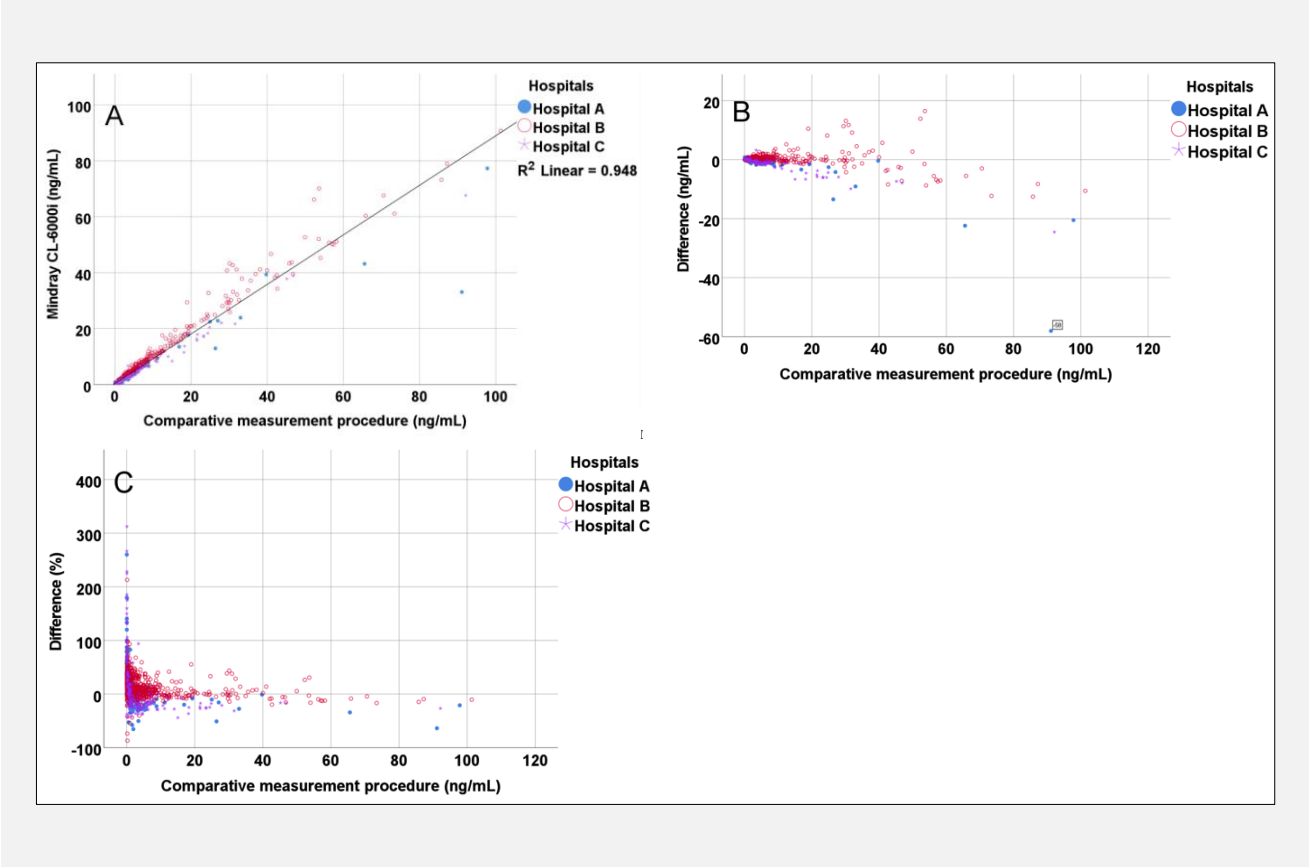

Figure 1. A - Comparison of serum PCT results between Mindray and Roche platforms (scatter plot), B - differences of serum PCT between Mindray and Roche platforms (scatter plot-bias), and C - value of differences of serum PCT between Mindray and Roche platforms (scatter plot of percentage).

the comparative concentration interval.

The data points in Figure 2C basically conform to the band distribution, and the difference was analyzed using the frequencies module of SPSS, with results shown in Table 3. After calculation, the bias fell on grade B of EP09-A3 (details omitted).

Figure 2D shows an approximately consistent spread of the percentage differences (constant coefficient of variation) across 10.0 - 100.0 ng/mL of the comparative concentration interval.

Since the data points in Figure 2D basically conform to the band distribution, the percentage difference was

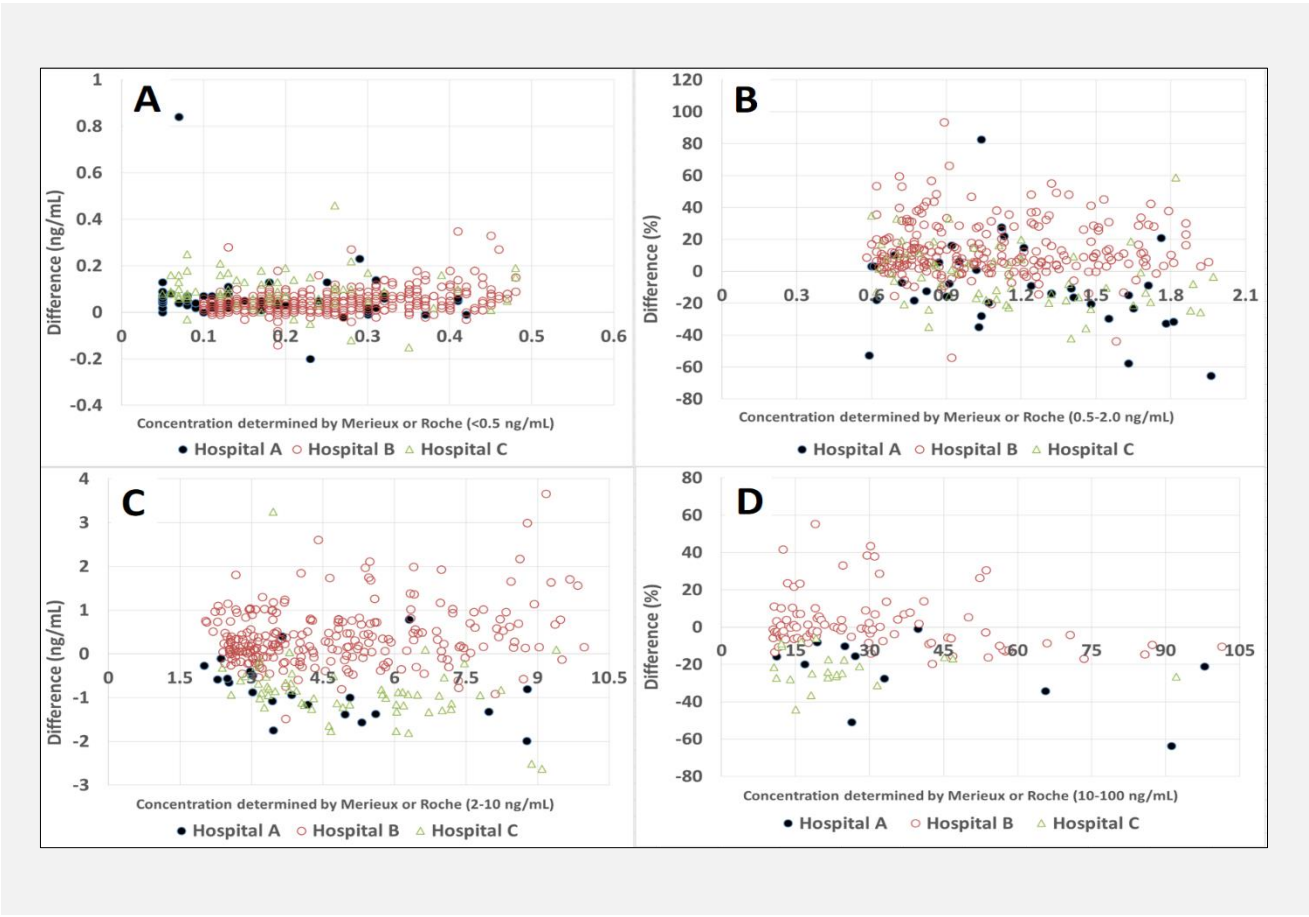

Figure 2. A - Value of differences of serum PCT at < 0.5 ng/mL level between Mindray and Roche platforms (bias plot), B - value of differences of serum PCT at 0.5 - 2 ng/mL level between Mindray and Roche platforms (bias percentage scatter plot), C - value of differences of serum PCT at 2 - 10 ng/mL level between Mindray and Roche platforms (bias plot), and D - value of differences of serum PCT at 10 - 100 ng/mL level between Mindray and Roche platforms (bias percentage scatter plot).

analyzed using the frequencies module of SPSS, with results shown in Table 4. After calculation, the bias fell on grade B of EP09-A3 (details omitted).

It is necessary to mention that Excel automatically selects the value of the Y-axis according to the maximum value of the negative value difference and the positive value difference, thus it was adapted without modification.

Estimation of bias based on difference or percentage difference

The acceptable bias was ± 0.055 (± 10.4%) at 0.53 ng/mL (low value) and ± 0.830 (± 9.0%) at 9.34 ng/mL (high value), based on CLSI EP21 - 2nd edition and Roche quality control materials as well as the Chinese Health Commission's "2017 Inter-laboratory Quality Assessment Plan". Based on these criteria, the biases listed in Table S3 are all acceptable.

Weighted least squares (WLS) regression analysis of PCT results from candidate method and comparative measurements (n = 1,325)

In Table S4, x_i represents the result from the comparative measurement, $\hat{Y}_i$ represents the estimated value of the candidate method at x_i, and $E\hat{Y}_i$ represents the mean of the $\hat{Y}_i$. The regression coefficient (b) (slope) and the intercept (a) of the linear regression reflect bias; among them, (b-1) defines the proportional bias and (a) defines the constant bias. Based on the results of outlier inclusion, the proportional bias is -1.79% (100 x (0.9821 - 1) (95% CI: -3.24%, -0.34%). The constant bias is 0.0613 ng/mL (95% CI of 0.0543 ng/mL, 0.0683 ng/mL). According to CLSI's guide series (such as EP21 - 2nd edition, EP09-A3, and EP09c, etc.), outliers require investigation into the cause. In addition, it is believed that large sample studies often produce some outliers (so EP21 - 2nd edition only introduces non-parametric statistical methods and cancels parametric statistical methods to cope with the existence of outliers). However, due to the nature of retrospective data analy-

sis, there was no outlier investigation at that time in this study.

Bias estimates ($\widehat{B}_c$) and relative bias estimates ($\widehat{B}_c$%) of PCT at a specific concentration (Xc)

Table S5 shows that the bias and bias % at the concentrations of 0.5 ng/mL and 2.0 ng/mL reached class B under the EP09-A3 guideline; meanwhile, they reached class A at the concentration of 10.0 g/mL (critical value). Thus, the biases listed in Table S6 are all acceptable.

DISCUSSION

Method comparison is commonly used in clinical laboratories [16]. The key element of method comparison is verification of trueness. Method trueness can be assessed either by using the CLSI EP15-A2, which defines the procedure of the verification of performance for precision and trueness, or by using the CLSI EP09-A3, which provides guidance on estimating the bias by comparison of measurement procedures using patient samples. The CLSI EP09-A3 can also be used to define the statistical procedures in the describing and analyzing of the data [17]. In this study, the CLSI EP09-A3 was used to quantify systematic measurement errors (bias) of serum PCT analytical results between the candidate method (Mindray CLIA) and comparative measurements (Roche Elecsys and VIDAS).

The influence of data distribution (scattering) on the quality of result comparison

To interpret a quantitative method comparison study, the correlation and regression methods, which usually use the concept of difference plots, are needed [18-20]. In our data set, there is one extreme outlier with a value of -58.03, as is displayed in Table S2, Figures 1A, 1B, and 1C. However, since outliers cannot be confirmed as erroneous results and the frequency of occurrence is also within the normal range, it is still used in the analysis. This extreme outlier is in the high concentration range, so it can have a large impact on the results of ordinary linear regression (OLR).

In the cases of Figure 1B and Figure 1C, it is impossible to obtain a band-type distribution of different data points over the full concentration range. Thus, the plot graphics were visually inspected according to different concentration segments, and the results were further analyzed with the difference or percentage difference that best matched the band-type distribution.

Regression analysis of analytical results of PCT from candidate method and comparative measurements

The bias and percentage bias reached grade B of EP09-A3 at 0.5 ng/mL (local infection) standard. At 2.0 ng/mL (systemic infection), they reached grade B of EP09-A3 of Roche internal quality control (IQC) standard. At 10.0 g/mL (critical value), they met the EP09-A3 grade A of the Roche IQC standard. Therefore, at each specific concentration in Table S5, the bias between the candidate method and the comparative measurements was acceptable and had good interchangeability.

Tables S4 and S5 indicate that the outlier (extreme) had little effect on the regression results, and neither excluding nor including outliers significantly changes the study conclusions as the sample size of this study is large; thus, the impact of individual outliers is limited, and weighted least squares regression can greatly weaken the impact of this outlier by reducing the weight of high concentration data.

It is worth to mention that our study did not record the concentration of interfering substances such as hemolysis and lipemia in the samples, but the instrument and reagent instructions of Mindray, Merieux, and Roche all list the robustness in the presence of interfering substances. Our results also show that there was a good comparability between their detection results without considering the concentration of interfering substances, indicating that interfering substances may not significantly affect our comparison results.

CONCLUSION

The bias between the PCT results of the candidate method (Mindray CLIA) and the comparative measurements (Roche and VIDAS) is acceptable. Therefore, the results of the three analytical platforms are comparable, and they can be mixed-used in the same institution.

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Ethical Approval and Consent to Participate:

This study was approved by the Institutional Review Board/Ethics Committee of Nanfang Hospital of Southern Medical University, Guangzhou Thoracic Hospital, and Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University (NFEC-2020-235, 2020 (No. 63), and SKSE C-KY-KS-2022-149, respectively). Patients' informed consents were obtained during the study. All methods were carried out in accordance with relevant guidelines and regulations. For patients younger than 16 years, the informed consent was obtained from parents or legal guardian(s).

Availability of Data and Materials:
All data from this study on which the conclusions rely are, for researchers who meet the criteria, available upon request from the corresponding authors.

Declaration of Interest:
All authors declared that they did not have any competing interests while conducting this study.

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