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Araştırma

The Need for Assay Harmonization: Carbohydrate Antigen 19-9 (CA 19-9) Assay Example

Harmonizasyon Gerekliliği: Karbonhidrat Antijen 19-9 (CA 19-9) Örneği

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ABSTRACT

Objectives: The carbohydrate antigen 19-9 assay is a widely used biomarker as a complement to other tests in the detection and follow-up of pancreatic, bile duct and colon cancer in high-risk individuals. The CA 19-9 assays often give different results with different methods. This study intended to characterize the harmonization problem in CA 19-9 assays using results obtained through external quality assessment.

Material and Methods: According to the 2018-External Quality Assessment program by the Randox International Quality Assessment Scheme, pooled serum specimens involving three levels of CA19-9 were analyzed. In addition, the results of four assays which are Abbott Architect, Beckman Access DXI600/800, Roche Cobas 6000/8000, and Siemens Advia Centaur XP/XPT/Classic were contrasted.

Results: Data from External Quality Assessment schemes demonstrate significant variation in CA 19-9 assay results obtained for the same specimen using different assays. The mean CA 19-9 evaluation of the peer groups differed for all four systems, and the interlaboratory CVs also varied. Generally, the highest peer groups mean values were gathered by employing the Abbott Architect system, which was followed by Siemens Centaur XP/XPT/Classic, while the lowest means were acquired utilizing the Roche Cobas system. Serum CA 19-9 assays also show a wide range in CV, which vary from 4.5-10.1%.

Conclusion: According to our findings, the harmonization of the CA 19-9 results obtained from four immunoassays have not been achieved yet. Systematic differences(different antibodies etc.) are still present among the most popular commercial methods for CA 19-9 measurement and may produce some difficulties in interpreting laboratory test results. Consequently, it is suggested that the assay or method used to identify CA 19-9 concentrations should be included in the laboratory result report.

Keywords: CA 19-9 Antigen, Harmonization, Immunoassay, Laboratory Proficiency Testing

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ÖZET

Amaç: CA 19-9, yüksek riskli kişilerde pankreas, safra kanalı ve kolon kanserlerinin tespiti ve takibinde diğer testlerin tamamlayıcısı olarak yaygın kullanılan bir biyobelirteçtir. CA 19-9 testinin farklı yöntemlerle ölçüm sonuçları arasında uyumsuzluklar bulunabilmektedir. Bu çalışmanın amacı CA 19-9 testinin harmonizasyon problemini ortaya koymaktır.

Gereç ve Yöntemler: Randox Uluslararası Kalite Değerlendirme Programı tarafından sağlanan 2018 yılına ait dış kalite değerlendirme programına göre, üç farklı seviyede CA 19-9 içeren dış kalite kontrol serum örneklerinin sonuçları değerlendirildi. Abbott Architect, Beckman Access DXI600/800, Roche Cobas 6000/8000 ve Siemens Advia Centaur XP/XPT/Classic cihazlarına ait sonuçlar karşılaştırıldı.

Bulgular: Dış kalite kontrol programından elde edilen verilere göre, aynı numune için farklı cihazlardan elde edilen CA 19-9 test sonuçları arasında bir uyum tespit edilememiştir. Dört sistemin her birine ait peer grup ortalamaları ve laboratuvarlararası CV'ler oldukça farklılık göstermektedir. Genel olarak, en yüksek peer grup ortalama değerleri Abbott Architect sistemi, bunu takiben Siemens Centaur XP/XPT/Classic sisteminden ve en düşük peer grup ortalamaları ise Roche Cobas sisteminden elde edilmiştir. Laboratuvarlararası % CV 4.5-10.1 arasında değişen geniş bir aralık göstermektedir.

Sonuç: Bulgularımıza göre, dört farklı sistem kullanılarak elde edilen CA 19-9 sonuçlarının harmonizasyonunun henüz sağlanamadığı görülmüştür. Farklı üreticilere ait kitlerle ölçülen CA 19-9 konsantrasyonları sistematik farklılıklar(farklı antikor kullanımı vb.) nedeniyle varyasyon gösterebilir. Sonuç olarak, CA 19-9 düzeyini ölçmede kullanılan kit/yöntemin laboratuvar sonuç raporunda belirtilmesinin faydalı olacağını düşünüyoruz.

Anahtar Kelimeler: CA 19-9, Harmonizasyon, İmmun testler, Dış Kalite Kontrol

INTRODUCTION

Achieving reliable, repeatable, and comparable laboratory test results is a significant issue in the field of laboratory medicine and can only be gained either by standardization or harmonization (1). The term "standardization" is used when results for a measurement are equivalent and traceable to the International System of Units (SI) through a high-order primary reference material and/or a reference measurement procedure (RMP). The harmonization of laboratory testing means that laboratory results are comparable within clinically meaningful limits among different laboratories using different measurement procedures and is generally used when results are equivalent, but usually implies there is no reference measurement procedure or certified reference material. The harmonization of laboratory testing means that laboratory results are comparable within clinically meaningful limits among different laboratories using different measurement procedures (2-5). Assessment for harmonization is particularly essential for serum tumor markers. Tumor markers can benefit the clinical monitoring of cancer patients, yet method-related differences in test results lead to misinterpretation and potentially affect the clinical decisions (6).

Carbohydrate antigen 19-9 (CA 19-9) is the most commonly used and only FDA-approved tumor marker for pancreatic cancer. It is a glycolipid antigen derived from mouse monoclonal antibody 1116-NS-19-9 (7-8). Serum levels of CA 19-9 can provide useful data for prognosis, overall survival, and the assessment of the response to systemic treatment as well as the prediction of post-operative recurrence (9). Radioimmunasay methods were used to measure serum CA 19-9 levels in the past, however various automated nonisotopic immunoassays have been developed currently, e.g., "sandwich" chemiluminescent immunoassays or electrochemiluminescence immunoassays (10,11).

CA 19-9 assays often present different results for the same sample among different measurement procedures. However, it is a significant fact that the results obtained from various analytical systems are accurate,

precise, and most importantly comparable (12). In the field of laboratory medicine, External Quality Assessment (EQA) schemes are one of the essential components of the quality management system of a laboratory, and it plays a vital role in the harmonization and standardization processes by ensuring the assessment and monitoring of the comparability of test results across different laboratories and over time (13-16). Even though quality control studies have been initiated, the disagreement of CA 19-9 results are commonly observed (11). The present study aimed to identify the problem of harmonization in immunoassays CA 19-9 testing using EQA data.

MATERIAL AND METHODS

This study was conducted in Clinical Biochemistry Laboratory of University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital and approved by the institutional ethics committee with the number of 116/01. According to the 2018 EQA program by the Randox International Quality Assessment Scheme (RIQAS, Randox Laboratories Ltd, United Kingdom) pooled serum samples including three levels of CA19-9 were evaluated. The RIQAS Immunoassay EQA program is created to monitor the performance of up to 55 immunoassay parameters, including therapeutic medicines, hormones, and tumor indicators. The EQA scheme follows a yearly cycle with 12 blinded samples collected at monthly intervals. The mean, standard deviation (SD), and coefficient variation (CV%) for each instrument, method, and all methods group are calculated for comparison. In RIQAS result evaluation report the multi method statistics summary part allows the laboratory professionals to review the performance of the techniques registered for each parameter. Reports from over 100 participating laboratories are available, and data in this study are obtained from the annual reports of the same clinical chemistry laboratories in 2018. In particular, data from widely used systems with more participants were included in the study. Thereby, the results of four assays that are Abbott Architect (Abbott Diagnostics, USA), Beckman Access DXI600/800 (Beckman Inc.,USA), Roche Cobas 6000/8000 (Roche Diagnostics, Indianapolis, IN) and Siemens Advia Centaur XP/XPT/Classic (Siemens Healthcare Diagnostics, USA) were compared. In addition, the assay peer group mean values and interlaboratory CVs for the CA19-9 measurements utilizing the four assays in the EQA scheme were analyzed. The clinical laboratory of the Diskapi Yildirim Beyazit Education of Research Hospital assayed the EQA samples with Beckman Access DXI 800 methods. Kit-specific information was taken from inserts provided by each IVD manufacturer and Substantial Equivalence Determination Decision Summary Food and Drug Administration (FDA) report.

RESULTS

Data from EQA schemes demonstrate significant variation in CA 19-9 assay results obtained for the same specimen using different assays. The mean CA 19-9 measurements of the peer groups were different for all four systems, and the interlaboratory CVs also varied (Table I). The mean CA 19-9 measurements of the peer groups for Level 1 sample issued through the RIQAS indicates that although results submitted may vary significantly (e.g from 17.971-229.429 U/ml) (about 12 times difference), for Level 2: 60.680-825.731 U/ml (about 13 times difference) and for Level 3:135.584-1925.496 U/ml (about 14 times difference). The highest peer groups mean values were gathered by using the Abbott Architect system, followed by Siemens Centaur XP/XPT/Classic while the lowest means were obtained utilizing the Roche Cobas system. Serum CA 19-9 assays also show a wide range in CV, which vary from 4.5-10.1%. The main characteristics of the four different CA 19-9 assay available on the market are presented in Table 2.

Table 1. Means and interlaboratory coefficient of variation for different CA19-9 assays in the 2018 EQA scheme.
Tablo 1. 2018 DKK şemasındaki farklı CA19-9 testleri için ortalamalar ve laboratuvarlar arası varyasyon katsayısı.

Systems	Level 1					Level 2					Level 3				
		n*	Mean (U/mL)	CV(%)**	Um***		n*	Mean (U/mL)	CV(%)	Um		n*	Mean (U/mL)	CV(%)	Um
Roche Cobas 6000/8000	Sample 1	258	19.794	5.1	0.08	Sample 2	312	69.366	4.70	0.23	Sample 3	322	138.595	4.6	0.44
	Sample 6	351	19.650	5.6	0.07	Sample 4	339	69.665	4.5	0.21	Sample 5	338	138.038	4.7	0.44
	Sample 8	283	18.005	6.5	0.09	Sample 7	279	61.325	4.7	0.22	Sample 9	333	135.584	4.7	0.44
	Sample 10	284	17.971	6.7	0.09	Sample 12	340	60.680	5.1	0.21	Sample 11	346	135.846	4.6	0.42
Beckman Dxl600/800	Sample 1	147	28.000	7.8	0.23	Sample 2	154	104.819	6	0.64	Sample 3	161	222.959	6.6	1.44
	Sample 6	161	28.064	5.5	0.15	Sample 4	160	106.051	6.1	0.64	Sample 5	158	230.202	6.5	1.49
	Sample 8	145	25.822	6.7	0.18	Sample 7	148	92.901	6.4	0.61	Sample 9	161	221.749	6.2	1.35
	Sample 10	157	25.262	5.1	0.13	Sample 12	168	93.123	5.7	0.52	Sample 11	161	220.191	5.2	1.12
Siemens Centaur XP/XPT/Classic	Sample 1	113	76.535	8.7	0.79	Sample 2	150	282.800	8.1	2.35	Sample 3	145	594.171	5.7	3.49
	Sample 6	141	72.668	9.4	0.72	Sample 4	138	270.181	6.3	1.82	Sample 5	139	586.181	5.1	3.18
	Sample 8	126	67.782	8.1	0.72	Sample 7	123	243.630	6.1	1.68	Sample 9	142	589.439	5.4	3.36
	Sample 10	124	68.184	8.6	0.66	Sample 12	137	258.507	7.2	1.98	Sample 11	141	617.159	7	4.56
Abbott Architect	Sample 1	133	228.726	7.2	1.78	Sample 2	169	822.324	7.8	6.15	Sample 3	164	1.925.496	10.1	18.89
	Sample 6	185	229.429	7.3	1.55	Sample 4	180	825.731	7.4	5.72	Sample 5	167	1.918,10	9.1	16.95
	Sample 8	152	209.451	7.6	1.61	Sample 7	154	735.095	6.7	4.99	Sample 9	153	1.878,25	9.3	17.67
	Sample 10	163	211.406	6.6	1.38	Sample 12	279	740.879	7.7	4.25	Sample 11	242	1.867,30	8.7	12.98

*n:participiant number of laboratories
**CV: coefficient of variation
***Um: Uncertainty of measurement

CA19-9: Carbohydrate Antigen 19-9,
EQA: external quality assurance,

Table 2. System characteristics of CA 19-9 assays.
Tablo 2. CA 19-9 testlerinin sistem özellikleri.

Systems	Measuring Range (U/mL)	Detection Limit (U/mL)	Total CV (%)	Cut-off (U/mL)
Roche Cobas 6000/8000	2-1000	<2	1.1-2.7	34(97.5%)
Beckman DXI 600/800	0.8-2000	<0.8	3-8.9	35(95%)
Siemens Centaur XP/XPT/Classic	1.2-700	<2	3.8-8.7	37(95%)
Abbott Architect	2-1200	<2	3.4-8.5	37(94.4%)

CA19-9: Carbohydrate Antigen 19-9, CV: coefficient of variation

Table 3. Summary of different CA 19-9 assay characteristics.
Tablo 3. Farklı CA 19-9 test özelliklerinin özeti.

System	Method	Antibody	Buffer	Tracer	Calibration	Predicate Device
Roche Cobas 6000/ 8000	Electrochemulinescence (ECLIA)	• Streptavidin-coated microparticles, • Biotinylated **monoclonal** CA 19-9 antibody (**mouse**) • Ruthenium complex labeled **monoclonal** CA 19-9 antibody(**mouse**)	Phosphate buffer	Rutenyum	The Elecsys CA 19-9 assay was standardized against the Enzymun CA 19-9 manufactured by Boehringer Mannheim Immunodiagnostics	Fujirebio Diagnostics CA 19-9™ RIA
Beckman DXI 600/800	Two-site immunoenzymatic ("sandwich") assay (chemiluminometric)	• Paramagnetic particles, coated with **goat polyclonal** anti-biotin antibody, bovine serum albumin, • **Mouse monoclonal** anti-CA 19-9 antigen-alkaline phosphatase (bovine) conjugate,bovine serum albumin, • **Mouse monoclonal** anti-CA 19-9 antigen-biotin conjugate, bovine serum albumin	Tris-buffered saline solution with BSA	Alkaline phosphatase	The Fujirebio CA 19-9 RIA assay was used to assign values to the primary reference calibrators	Fujirebio Diagnostics CA 19-9™ RIA
Siemens Centaur XP/XPT/ Classic	Two-step sandwich immunoassay (chemiluminometric)	• **Monoclonal mouse** anti-CA 19-9 antibody covalently coupled to paramagnetic particles • **Monoclonal mouse** anti-CA 19-9 antibody labeled with acridinium ester	-	Acridinium ester	-	Fujirebio Diagnostics CA 19-9™ RIA
Abbott Architect	Two-step immunoassay (using Chemiluminescent Microparticle) Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex)	• Microparticles coated with **monoclonal mouse** anti-116-NS-19-9 antibodies in citrate buffer • Acridinium-labeled **monoclonal mouse** anti-1116-NS-19-9 antibody conjugate in phosphate buffer	Citrate buffer Phosphate buffer	Acridinium ester	The ARCHITECT® CA 19-9™XR Calibrators were standardized against the Fujirebio Diagnostics, Inc. CA 19-9 reference preparation.	Fujirebio Diagnostics CA 19-9™ RIA

CA19-9: Carbohydrate Antigen 19-9, RIA: radioimmunoassay

DISCUSSION

The standardization and harmonization of laboratory tests is necessary for the production of globally interchangeable test results and helpful to realize to what extent method-related differences are likely to present (1,6). The International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR) was founded to offer a coordinated process for organizing global efforts to harmonize clinical laboratory test results. For this purpose, a website portal is available at www.harmonization.net, which gives information on the status of harmonization or standardization of measurands and further information concerning the medical implications. Harmonization status of CA 19-9 is specified as "Needed" and medical impact of harmonization for CA 19-9 is specified as "High" on this website (17).

The comparability of methods and monitoring of the harmonization process for the analytical phase can be monitored by EQA schemes. It is extremely important that commutable EQA-material is used so that performance between methods can be assessed (1, 18). Commutability is a feature of reference materials, which refers to those that have the same inter-assay relationships as clinical samples (19). A commutable EQA sample acts similarly to a native patient sample with the same numeric relationship between measurements procedures as observed for a panel of patient samples. Unfortunately, commutable EQA programs are rare due to a lack of reference measurement processes, absence of verified reference materials, and inability to prepare commutable samples (15).

This study aimed to identify the harmonization issue in immunoassays for CA19-9 testing. The results from the EQA-data show that the harmonization of CA19-9 assays is in many cases far from being optimized, and the comparability of results and monitorization of patients are only possible when using the same method over a long period of time. The most applicable parameter to determine the equivalence of results (the degree of standardization/ harmonization) is the overall interlaboratory CV. The mean lowest CV% value was obtained using the Roche Cobas system, and the mean highest CV% value was obtained using the Abbott Architect system for three-level control. According to our findings, between-method agreement and harmonization of the CA 19-9 results obtained by utilizing 4 immunoassays has not yet been achieved as illustrated by EQA data.

Since there are differences in assay methodologies, antibodies utilized, epitope specificity, and reagent specificity; the CA 19-9 concentration in a given specimen obtained by using various vendor's assays can show alterations. Based on the instructions of manufacturers, the solid-phase antibodies and labeled tracer antibodies of the three immunoassays used in this study (Abbott, Roche, and Siemens) were mouse monoclonal anti-CA 19-9 antibodies, whereas the solid-phase antibody and labeled tracer antibody of the Beckman assay were goat polyclonal antibody and mouse monoclonal antibody, respectively (Table III). These inconsistencies might be due to the absence of an international reference standard for various manufacturers to utilize when calibrating their kits. The carbohydrate structure of the CA 19-9 molecule is very heterogeneous (6). Therefore, the value assignment of the standard is complex, and it is quite impossible to generate a glycoprotein standard that is similar to the circulating form (14). It is also apparent that the differences cannot be attributed solely to the use of different antibodies because significant differences can be detected even between assays conducting the same monoclonal antibody: the variables involved in an immunoassay are numerous (e.g., dilutions, incubation times, reaction kinetics) and they all combine to produce a result that varies significantly even in the presence of the reference standard (20).

The comparison of various assays for CA19-9 has been intensively investigated in recent years. Stern P. et al. (21) compared six routinely used immunoassay kits: Architect i2000 and AxSYM, Elecsys 2010, ELSA, Immulite 1 and IRMA-mat. In order to assess the comparability of results, 81 normal and pathological patient samples were used, and they found that systematic differences among the measurement systems are large. Hotakainen K et al (22) compared three CA 19-9 assays: Abbott i2000 Architect (CA 19-9XR assay), Roche Elecsys 2010 and Bayer Immuno 1 analyzers. They reported that the three CA 19-9 assays present quite variable results especially at low and moderately elevated concentrations. The results obtained from the Architect CA 19-9XR assay are found to be significantly lower than those with the other assays in patients with benign conditions and lower than with the Elecsys in apparently healthy controls, while the concentrations in cancer patients are more similar with all assays analyzed. In another study, Passerini R. et al. (23) compared the results of two commercial immunoassays (Abbott ARCHITECT i2000 and Roche cobas 410). They found that those two immunoassays are comparable in terms of diagnostic accuracy and had significant correlation but are not interchangeable.

In conclusion, the CA 19-9 results obtained through the 4 immunoassays have not been harmonized yet. Systematic differences are still present among the most popular commercial methods for CA 19-9 measurement and may produce some difficulties in interpretation of laboratory test results. Physicians need to be aware of the inconsistency of different commercial methods/devices and assays so that they can critically interpret the test results reported by different laboratories. Consequently, the levels of CA19-9 measured using different assays may show significant differences, and patients should be monitored with the same method, as method-related differences in results may adversely.

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