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Clinical performance characteristics of a laboratory test. A practical approach in the autoimmune laboratory

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ABSTRACT

Background: This review aims to make laboratory professionals and clinicians more familiar with the basic concepts that deal with the clinical performance characteristics of a laboratory test.
Content: Basic measures of the clinical performance characteristics of a laboratory test, such as sensitivity, specificity, likelihoods, likelihood ratio, post-test probability, odds, Bayes theorem, and receiver–operator characteristic curve are explained. The concepts are illustrated with examples (anti-CCP antibodies) that are worked out in a spreadsheet.
Summary: This review seeks to provide laboratory professionals and clinicians with a better understanding of the clinical performance characteristics of a laboratory test and of evidence-based laboratory medicine. The manuscript emphasizes a strong link between clinical statistics and evidence-based laboratory medicine.

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1. Introduction

Concepts of evidence based laboratory medicine are increasingly utilized in routine clinical and laboratory practice. It is my experience, however, that some basic concepts of evidence based laboratory medicine, such as likelihood ratios, are not fully comprehended by laboratory professionals and clinicians.

In clinical practice the question clinicians want to answer by ordering a laboratory test is: "What is the probability of a patient having (or not having) disease X when the laboratory test is positive (or negative)?" and not the question: "What is the probability of a patient having a positive (or negative) test result if they have disease X?".

This review aims to make laboratory professionals and clinicians more familiar with the basic concepts that deal with clinical performance characteristics of a laboratory test.

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Likelihoods of anti-CCP test result given the disease status

	RA	non RA
anti-CCP+	0,67	0,05
anti-CCP-	0,33	0,95

Pre-test probability of disease status

RA	non RA
0,1	0,9

Joint probabilitity of disease status and test results

	RA	non RA	Unconditional
anti-CCP+	0,067	0,045	0,11
anti-CCP-	0,033	0,855	0,89
	0,1	0,9	1

Post-test probabilities of disease status

	RA	non RA	
anti-CCP+	0,598	0,402	1
anti-CCP-	0,037	0,963	1

Likelihoods of test result given the disease status

	D	ND
T+	*P* (T+ ID)	*P* (T+ IND)
T-	*P* (T- ID)	*P* (T- IND)

Pre-test probability of disease status

D	ND
P (D)	*P* (ND)

Joint probabilitity of disease status and test results

	D	ND	Unconditional
T+	*P* (T+ ID)**P* (D)	*P* (T+ IND)**P* (ND)	*P* (T+) = *P* (T+ ID)**P* (D) + *P* (T+ IND)**P* (ND)
T-	*P* (T- ID)**P* (D)	*P* (T- IND)**P* (ND)	*P* (T-) = *P* (T- ID)**P* (D) + *P* (T- IND)**P* (ND)

Post-test probabilities of disease status

	D	ND
T+	*P* (D IT+)	*P* (ND IT+)
T-	*P* (D IT-)	*P* (ND IT-)

Post-test probabilitiesof disease status *

	D	ND
T+	*P* (T+ ID)**P* (D)/*P* (T+)	*P* (T+ IND)**P* (ND)/*P* (T+)
T-	*P* (T- ID)**P* (D)/*P* (T-)	*P* (T- IND)**P* (ND)/*P* (T-)

* divide joint probability by unconditional probability

Fig. 1. Calculation of post-test probabilities based on Bayes' rule. The likelihoods are from Ref. [2]. The method for calculation has been adapted from Refs. [4,5]. T: test result status, D: disease status.

2. Sensitivity, specificity, and likelihoods

The clinical sensitivity of a laboratory assay reflects the fraction of diseased cases that a laboratory test correctly predicts [1]. For example, a recent meta-analysis reported that anti-cyclic citrullinated peptide (CCP) antibodies had a pooled sensitivity of 67% (95% confidence interval (CI), 62% to 72%) for rheumatoid arthritis (RA) [2], which means that anti-CCP antibodies were found in 67% of RA patients. The diseased cases are diagnosed according to a clinical reference standard. For RA, diagnostic criteria have been established by the American College of Rheumatologists (ACR) [3].

The clinical specificity of a laboratory assay reflects the fraction of individuals with absence of disease that the assay correctly predicts [1]. For example, a recent meta-analysis reported that anti-CCP antibodies had a specificity of 95% (95CI, 94% to 97%) [2], which means that 95% of individuals without the disease (controls) tested negative for anti-CCP antibodies. Preferably, the control group should include individuals with diseases related to the principal disease a

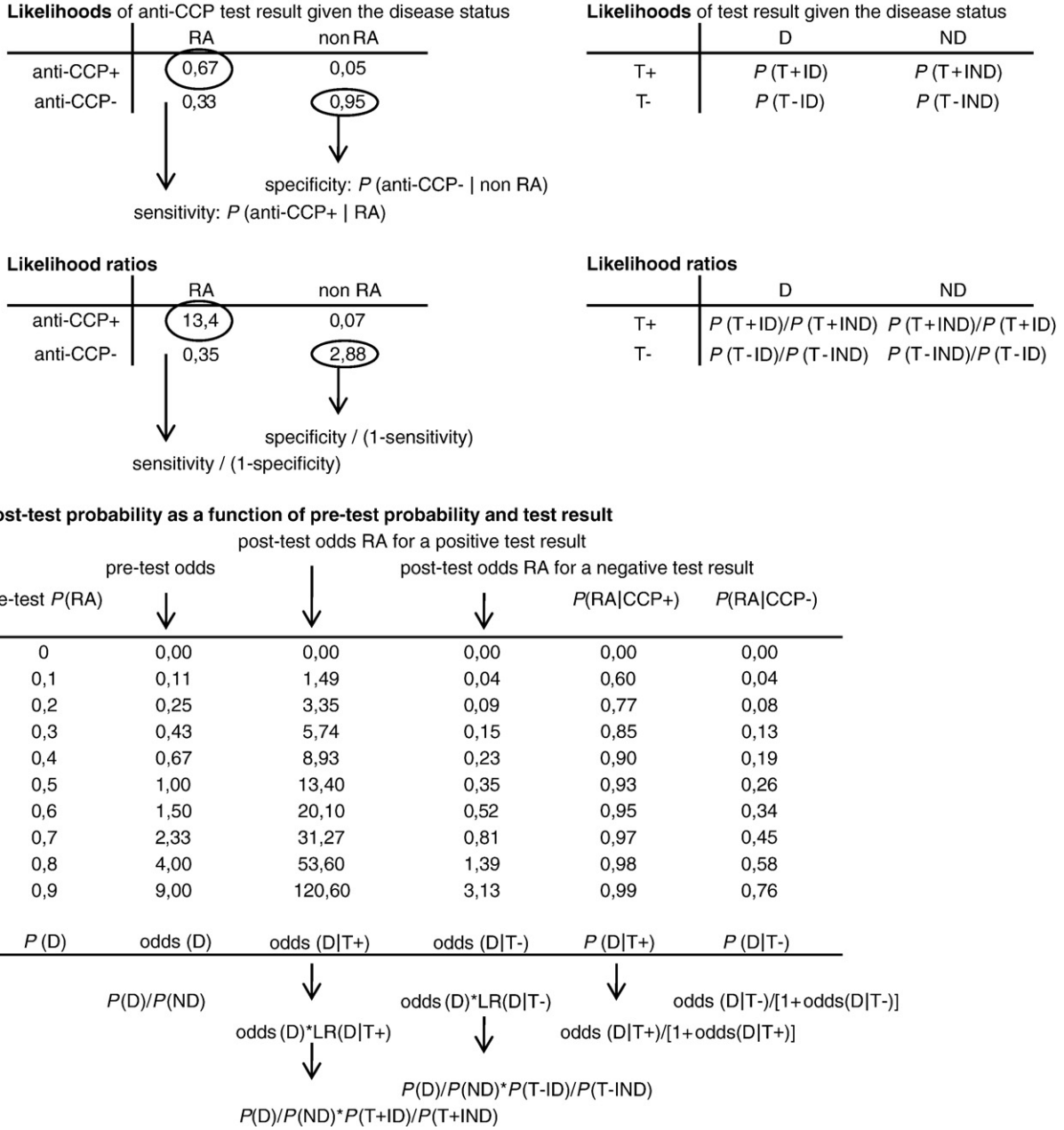

Fig. 2. Calculation of post-test probabilities based on the likelihood ratio. The likelihoods are from Ref. [2]. T: test result.

laboratory test has been shown to be useful in identifying, but who lack this principal disease (i.e. "diseased control group"). For example, in the anti-CCP study, the control group included patients with rheumatic diseases other than RA. The rationale for this type of selection of control group individuals is that the diagnostic specificity determined in a group of "healthy" individuals may be higher than the specificity determined in diseased controls.

In statistical terms, the sensitivity corresponds to the conditional probability of a positive test result (T) in patients with the disease (D). This is denoted by $P(T+|D)$. The specificity corresponds to the conditional probability of a negative test result in patients without the disease (ND). This is denoted by $P(T-|ND)$. With a dichotomous test, there are four conditional probabilities of test results given the disease status: $P(T+|D)$, $P(T-|D)$, $P(T+|ND)$, and $P(T-|ND)$. These conditional probabilities are called likelihoods. The likelihoods of anti-CCP test results given the disease status (RA or diseased control) are illustrated in Figs. 1 and 2 (data for sensitivity and specificity are from Ref. [2]).

3. Calculation of post-test probabilities based on Bayes' rule

If the pre-test probability of the disease [$P(D)$] is known, then the post-test probability [e.g. $P(D|T+)$] can be calculated based on the likelihoods and the pre-test probability by application of Bayes' rule. The terms 'pre-test' and 'post-test' refer to the condition before and after testing [4,5].

$$Bayes' \ rule: P(D|T) = P(T|D)*P(D) / (P(T|D)*P(D) + P(T|ND)*P(ND))$$

$$P(D|T) = P(T|D)*P(D) / P(T)$$

Fig. 1 llustrates application of Bayes' rule using the likelihoods of anti-CCP and assuming a pre-test probability of RA of 10%. The likelihoods as well as the pre-test probabilities are given. The joint probabilities are calculated by multiplying the likelihoods with the pre-test probabilities [4,5]. The sums of the joint probabilities are the unconditional probabilities. The post-test probabilities are calculated by dividing the joint probabilities by the unconditional probabilities [4,5].

Post-test probabilities can be calculated for several pre-test probabilities (ranging between 0 and 1). Fig. 3 panel A illustrates how the post-test probability for RA of a positive and negative anti-CCP test result depends on the pre-test probability. For example, the post-test probability of a positive anti-CCP test result is 0.6 and 0.9 when the pre-test probability is, respectively, 0.1 and 0.4. It may be difficult to estimate the pre-test probability in an individual patient. A patient presenting with knee pain in primary care (who does not meet other ACR criteria) will have a low pre-test probability, whereas a patient seen in a rheumatology clinic who meets other ACR criteria will have a high pre-test probability for RA [2]. Prediction scores to predict the risk (probability) of RA have been developed [6].

A similar approach can be used to calculate the positive and negative predictive value. The positive predictive value tells how often a diseased patient (e.g. RA patients) actually has the disease given a positive test result [1]. The negative predictive value tells how likely it is that a control individual (diseased control) does not have the disease given a negative test result [1]. The positive and negative predictive values depend on the prevalence of the disease in a defined population. The predictive values can be calculated based on the disease prevalence in a population and the likelihoods of the assay by application of Bayes' rules (as illustrated above).

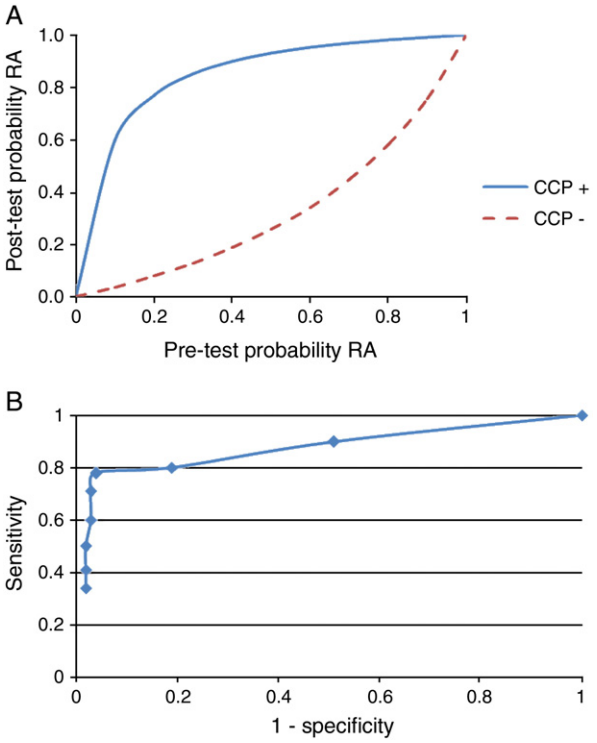

Fig. 3. Panel A. Post-test probability as a function of pre-test probability and anti-CCP test result. Panel B. ROC curve. Data are for the anti-CCP assay from Phadia (obtained from Ref. [10]).

4. Calculation of post-test probabilities based on likelihood ratios

The likelihood ratio of a specific test result for a disease is the likelihood of the test result in diseased individuals divided by the likelihood of the test result in diseased control individuals. The likelihood ratios of anti-CCP for RA and disease controls are illustrated in Fig. 2. The positive likelihood ratio corresponds to *sensitivity / [1 − specificity]*. The negative likelihood ratio corresponds to *specificity / [1 − sensitivity]*.

Likelihood ratios can be used to calculate post-test probabilities using the following formula (which is a variant of Bayes' theorem):

$$Post\text{-}test \ odds = pre\text{-}test \ odds \times likelihood \ ratio$$

$$P(D|T) / P(ND|T) = P(D) / P(ND)*P(T|D) / P(T|ND)$$

The conversion of probabilities to odds is done as follows:

$$Odds = probability / (1 - probability)$$

The conversion of odds to probabilities is done as follows:

$$Probability = odds / (1 + odds)$$

Fig. 2 illustrates the calculation of post-test probabilities based on likelihood ratio and pre-test odds for anti-CCP.

The likelihood ratio provides an estimation of whether there will be significant change in pre-test to post-test probability of a disease given the test result [7]. A likelihood ratio of 1 implies that there will be no difference between pre-test and post-test probability [7]. Likelihood ratios >10 or <0,1 indicate large, often clinically significant differences, likelihood ratios between 5 and 10 and between 0,1 and 0,2 indicate modest clinical differences, likelihood ratios between 2 and 5 and between 0,5 and 0,2 indicate small but potentially relevant clinical differences, likelihood ratios between 1 and 2 and between 0,5 and 1 indicate small differences (rarely clinical significant) [7]. Nomograms to help with the calculation of post-test probabilities based on likelihood ratios have been developed [8].

5. Post-test probabilities for test result intervals

The clinical significance of a slightly elevated test result might be different from the clinical significance of a highly elevated test result. Therefore, it is relevant to define the clinical performance characteristics of a laboratory test for several test result intervals. In a recent study, we demonstrated how the likelihood ratios and post-test probabilities of anti-CCP antibodies depend on the antibody concentration [9]. Fig. 4 illustrates the calculations (based on Bayes' rule) for several anti-CCP test result intervals (for the data presented in Ref. [9]). The likelihood ratio for anti-CCP were 0.23, 4.5, and 27.7 for <7 units/mL, 7–25 units/mL, and >25 units/mL, respectively [9]. Such knowledge helps with the interpretation of a specific test result. For example, a negative test result cannot be used to certainly exclude RA, as a likelihood ratio of 0.23 indicates only a rather small (but relevant) difference in pre-test to post-test probability. A low positive rest result for anti-CCP also indicates only a small (but relevant) difference in pre-test to post-test probability (likelihood ratio of 4.5). By contrast, a highly elevated anti-CCP value (likelihood ratio of 27.7) will significantly affect post-test probability. Consider a patient for whom the clinician estimates that the pre-test probability for RA is 0.1. For example, a female patient, 50 years old, with recent onset undifferentiated arthritis [intermittent asymmetric tender and swollen small joints ($n = 5$) of the hands (CRP: 10 mg/L)]. If the result of the anti-CCP test is negative, then the post-test probability will be 0.025. If the result is of the anti-CCP test is between 7 and 25 units/mL, then the post-test probability of RA will be 0.33. If the anti-CCP test result is >25 units/mL, then the post-test probability for RA will be 0.75. This example clearly illustrates how test result specific likelihood ratios (and probability data) can be used to add value to a laboratory test result. Clinical laboratories should consider to provide likelihoods ratios for test result intervals [9]. Likelihood ratios for test result intervals are more informative than a single cut-off value.

6. Receiver–operator characteristic curve

Sensitivity and specificity depend on the cutoff value. Increasing the cutoff value decreases the sensitivity and enhances the specificity, whereas decreasing the cutoff value enhances sensitivity and decreases the specificity. For example, the sensitivity of the Phadia anti-CCP assay was 1, 0.9, 0.8, 0.78, 0.71, 0.6, 0.5, 0.41, 0.34 for a cutoff of 0.3, 1.8, 3.1, 7, 25, 46, 125, 215, 340 units/mL, respectively (data are from Ref. [10]). The specificity was, respectively, 0, 0.49, 0.81, 0.96, 0.97, 0.97, 0.98, 0.98, 0.98. When preparing a receiver–operator characteristic (ROC) curve, sensitivity at all test values is plotted typically on the y-axis, while corresponding specificity values are plotted on the *x*-axis. The area-under-the-curve (AUC) is a relative measure of the diagnostic accuracy (i.e., the diagnostic sensitivity and

Likelihoods of anti-CCP test results given the disease status

	RA	non RA
CCP < 7 units/mL	0,23	0,96
CCP 7-25 units/mL	0,07	0,02
CCP > 25 units/mL	0,71	0,03

Likelihood ratios

	RA	non RA
CCP < 7 units/mL	0,24	4,25
CCP 7-25 units/mL	4,48	0,22
CCP > 25 units/mL	27,67	0,04

Pre-test probability of disease status

RA	non RA
0,1	0,9

Joint probability of disease status and test results

	RA	non RA	Unconditional
CCP < 7 units/mL	0,023	0,863	0,89
CCP 7-25 units/mL	0,007	0,014	0,02
CCP > 25 units/mL	0,071	0,023	0,09
	0,1	0,9	1

Post-test probabilities of disease status

	RA	non RA
CCP < 7 units/mL	0,025	0,975
CCP 7-25 units/mL	0,333	0,667
CCP > 25 units/mL	0,755	0,245

Fig. 4. Calculation of post-test probabilities for more than two test result intervals. The likelihoods are from Ref. [9].

specificity of the test) of a test [1,11] and allows comparison of the diagnostic accuracy of different tests. AUC values range between 0 (a test with no clinical utility) and 1 (a "perfect" test: sensitivity = specificity = 100% at all test cutoff values). The clinical value of laboratory tests with AUC values between 0 and 0.5, 0.5–0.7, 0.7–0.9, or >0.9 is none, limited, moderate, and high, respectively. The AUC for the Phadia anti-CCP assay was 0.88 [10]. The ROC curve for anti-CCP is shown in Fig. 3 panel B.

7. Conclusion

In this brief overview of various indices of laboratory test clinical diagnostic performance, I have explained the importance of such test concepts and calculations as sensitivity, specificity, likelihood, likelihood ratios, pre- and post-test probability, odds, Bayes theorem, and ROC curves. These concepts were illustrated using data from a study of CCP antibody levels in discriminating between RA and diseased control individuals. I have shown how likelihood ratios can enhance the clinical interpretation of a laboratory test result. We are currently using likelihood ratios by including them in the laboratory report for anti-CCP.

The aim of this overview was to give laboratory professionals and clinicians a better insight into the basic concepts of evidence-based laboratory medicine related to the evaluation of the clinical performance characteristics of laboratory tests.

Take-home messages

- Likelihood ratios can enhance the clinical interpretation of a laboratory test result.
- The likelihood ratio of a specific test result for a disease is the likelihood of the test result in diseased individuals divided by the likelihood of the test result in control individuals.
- Likelihood ratios can be used to calculate post-test probabilities.
- Likelihood ratios can be calculated for test result intervals.

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Antineuronal antibodies in Parkinson`s disease

The autoimmune origin of the postinfectious movement disorders, such as Sydenham's chorea has been related with the presence of certain autoantibodies such as the antineuronal antibodies (ANAs). However, their relevance in other movement disorders–in the absence of infectious triggers–remains largely unclear. In addition, the autoimmune origin of neurodegenerative diseases is even more a big source of uncertainty and discussion. In a recent study performed by **van de Warrenburg BP. et al. (Mov Disord 2008; 23:958–63**), the frequency of ANAs in idiopathic Parkinson's disease (IPD) and an exploration on whether a specific phenotype is likely associated with the presence of ANAs was developed. They recruited 76 IPD patients, 9 patients with genetic Parkinsonism, and 10 with one of the Parkinson-plus syndromes. A comprehensive clinical review was assessed in overall patients. In addition, 50 patients with non-extrapyramidal neurological disease and 30 healthy blood donors were employed as control populations. Blood samples were tested for the presence of ANAs with Western blotting, using recombinant proteins of the three putative antigens (aldolase C, neuron-specific enolase, and pyruvate kinase M1). They found these antibodies in 11.8% of the 76 IPD patients, which differed significantly from healthy controls (0%, P = 0.043), but non-significantly from patients with genetic Parkinsonism (11.1%), with a Parkinson-plus syndrome (10%), or from neurological disease controls (4%). With respect to relevant disease characteristics, IPD patients with or without ANAs were indistinguishable, except for atypical disease features (mainly early falls or freezing and marked Pisa syndrome), which were more frequent in the ANA-positive IPD group. They concluded that ANAs appear not to play a role in the majority of patients with IPD, but might be relevant in the pathogenesis of IPD with atypical features.