

Rational use of laboratory tests in dermatology

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Background

Laboratory and *in vivo* test results greatly influence diagnosis and treatment. Therefore, it is essential to select appropriate tests in the diagnostic process and keep in mind that each test can produce false positive and false negative results. The selection of an optimal test requires a thorough understanding of the concepts delineating the efficacy of a diagnostic test such as sensitivity, specificity, positive and negative predictive value and likelihood ratios for a positive and negative test.¹

While accurate interpretation of laboratory tests often depends on the use of statistical concepts we learned during medical training, many of us find it difficult, for different reasons, to incorporate these principles into a busy practice. We need to be aware of the fact that without correct interpretation laboratory results are hardly meaningful. Laboratory mistakes are not yet defined as diagnostic errors, but they contribute significantly to the thousands of medical errors that happen every year.²

Overuse of laboratory tests is problematic. Because “normal range” for test results are based on statistical analysis, as many as 5% of patients in a standard distribution fall outside the range.³ It is important to order only the tests we really need as extra testing automatically means more false positive results. The risk of false positive results increases up to 40% when a patient is exposed to ten unnecessary laboratory investigations.⁴

Unfortunately, such misuse of laboratory tests is widespread. In a systematic review of 44 eligible published studies measuring inappropriate laboratory utilization in the light of methodological criteria, there was widespread prevalence but large variations in the estimates of inappropriate laboratory use (4.5%–95%).⁵ Though data obtained from this review is two decades old, more recent

studies have hardly shown any improvement in this regard. In a study carried out in the intensive care unit of a general hospital in Brazil, 41% ($n = 719$) of the 1,750 lab tests ordered were found to be unnecessary.⁶ In a South African study, in 59.1% of cases, the test results were incorporated into the physicians’ management plan for the patient, that is, in 41.9% of cases the results had no bearing on further management of the patients.⁷ Effective use of lab tests is really the key as there is overwhelming evidence that such use significantly improves diagnosis and treatment at every setting and every level of medical care.⁸

With the rapidly increasing volume of medical research being conducted and clinical laboratory tests being developed, physicians are challenged increasingly on how best to integrate clinical and laboratory evidence in making decisions about the day-to-day care of their patients.⁹ No sure method exists for eliminating biases in medical decision making, but there is some evidence that the adoption of an evidence-based medicine approach or the incorporation of formal decision analytic tools can improve the quality of physicians’ reasoning.¹⁰

In one of the rare studies conducted among patients in dermatology outpatient clinics who had been advised laboratory tests, the cognitive or psychological effect of the examinations were tested according to the hypothesis that performing laboratory tests increases the patients’ fear of morbidity and mortality, and therefore, has a positive effect on the patients’ attitude toward the doctors’ recommendations and willingness to accept them. Patients who had undergone laboratory tests one week before the survey had a tendency to show even lower positive attitude toward the doctor’s recommendations and less intention to follow the recommendations. In contrast to the hypothesis that also happens to be the conventional wisdom, performing laboratory tests does not subliminally increase patients’ fears or anxieties about their disease or their compliance with doctors’ recommendations.¹¹

This editorial will seek to refresh the relevant statistical concepts. We shall also discuss certain tests commonly employed by dermatologists in day-to-day practice and exemplify how the test results are reliable only if comorbidities, pre- and post-test

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probabilities and clinical context are carefully considered. Thus, we hope a rational understanding of the laboratory tests that we employ may develop.

Key Concepts

What are the rational indications of performing a test? In a nutshell, these are:

- Diagnosis of disease
 - Occult disease
 - Early diagnosis
 - Differential diagnosis
- Prognosis of disease
- Staging of disease
- Estimating activity of disease
- Detecting recurrence
- Monitoring effect of therapy
- Prediction of response to therapy
- Prediction of drug toxicity
- Monitoring for adverse drug reactions
- Genetic counselling
- Medicolegal problems (e.g., paternity).

Thus, laboratory or *in-vivo* tests are an integral part of our diagnostic techniques. However, it is not mandatory to order tests for every patient, irrespective of the clinical situation.

If we review strategies of clinical diagnosis, we find that a diagnosis may be made by specific clinical features alone (viz., erythema multiforme), lab tests alone (diabetes mellitus), clinical features aided by histopathology (e.g., vasculitis, panniculitis and sundry other inflammatory and neoplastic dermatoses), clinical features aided by lab test (e.g., connective tissue diseases), clinical criteria alone (atopic dermatitis according to UK Working Party’s diagnostic criteria) and by means of clinical and lab criteria (atopic dermatitis according to Hanifin and Rajka’s criteria).

In an ideal world, an ideal laboratory test should have the following characteristics:

- Positive in every patient with the disease
- Negative in every patient without the disease
- Positive from an early or very early stage
- Vary synchronously with corresponding disease activity.¹²

As we live in a world far from ideal, we need to ask some questions whenever we order a lab test. These are:

1. What is the reason for the test?
2. What are the consequences of not ordering the test?
3. How is the test result interpreted:
 - a. In patients with the disease, what proportion tests positive?

- b. In patients without the disease, what proportion tests negative?
 - c. If a patient tests positive, what is the probability of having the disease?
 - d. If a patients tests negative, what is the probability of not having the disease?
4. On the basis of our interpretation, is the test adequate for differentiating people who are diseased from the nondiseased?
 5. Whether the test result will change the diagnosis or prognosis or therapy?
 6. Will the result provide a better understanding of the disease process that is going on in the patient?
 7. How will the test results affect the patient’s life?¹³

In good clinical practice, it is necessary to realize that every test can be a source of false positive and false negative results. This is expressed as a classic 2 × 2 table and is interpreted as such [Table 1]. The risk of such errors is usually described using indicators of sensitivity and specificity. Table 2 provides the definitions of terms useful in determining the effectiveness of laboratory tests using those indicators.

Rational Uses of Diagnostic Tests: A Few Examples

Here, we shall discuss a few examples of the rational use of commonly used *in-vitro* tests. In all these cases, the test results are reliable only if comorbidities, pre- and post-test probabilities, and clinical context are carefully considered.

How to interpret abnormal liver function tests for patients on methotrexate therapy

Abnormal liver function tests should never be presumed to be caused by methotrexate. The available evidence indicates that methotrexate-related liver adverse events are rarely serious, particularly in the short term, whereas many other causes of abnormal liver function tests may be serious. An evaluation for other potential causes should follow identical pathways and similar rigor to that applied to a patient who is not taking methotrexate.¹⁴

If after exhaustive investigation no cause other than methotrexate is identifiable, the treatment approach recommended in guidelines depends on the degree of transaminase elevation. The baseline transaminase levels prior to methotrexate institution are also important; a previously elevated transaminase level that has not changed following institution of methotrexate is unlikely to need further intervention. The threshold for immediately interrupting methotrexate use differs by the respective guideline; however, levels greater than three times the upper limit of normal are often used.¹⁵ Persistent lower degree elevations may also require intervention particularly if the trend is for a progressive increase in

Table 1: Possible results of a diagnostic test

	Diseased	Nondiseased	
Positive test	True positive (TP)	False positive (FP)	Total number of positive tests (TP + FP)
Negative test	False negative (FN)	True negative (TN)	Total number of negative tests (FN + TN)
	Total number of cases who are diseased (TP + FN)	Total number of cases who do not have the disease (FP + TN)	Total number of cases (TP + FP + FN + TN)

TP (true positive): The test is positive, the patient is diseased, TN (true negative): The test result is negative, the patient does not have the disease, FP (false positive): Test result positive, the patient is not having the disease, FN (false negative): Test result negative and the patient is having the disease

Table 2: Definitions of terms useful in determining the effectiveness of a laboratory test

Term	Short definition	Formula
Sensitivity	The ability of the test to detect people actually diseased	$\frac{TP}{(TP + FN)} \times 100\%$
Specificity	The ability of the test to detect people who are really non-diseased	$\frac{TN}{(TN + FP)} \times 100\%$
PPV	Percent of patients with positive test result who are actually diseased	$\frac{TP}{(TP + FP)} \times 100\%$
NPV	Percent of patients with negative test result who actually do not have the disease	$\frac{TN}{(TN + FN)} \times 100\%$
Diagnostic accuracy	Percent of patients properly diagnosed by the test as either having the disease or not having it	$\frac{(TP + TN)}{(TP + FP + FN + TN)} \times 100\%$
LR+	The ratio of the probability of having a positive test result in a person who is diseased and the probability of having a positive test result in a person who is not diseased	$\frac{\text{Sensitivity}}{1 - \text{Specificity}}$
LR-	The ratio of the probability of having a negative result in a person who is diseased and the probability of having a negative result in a person who is not diseased	$\frac{1 - \text{Sensitivity}}{\text{Specificity}}$
Cut-off value	In the case of test results having continuous values, the point above which we consider the result to be of pathological significance	Determined by means of ROC curves
Clinical relevance	The significance of the result for the patient's current disease (problem)	
Reproducibility (repeatability)	Compatibility of independent results from the same material obtained with the same analytical method in identical conditions (the same analyst, the same laboratory, the same apparatus, etc.)	
Reproducibility (interlaboratory agreement)	Compatibility of independent results from the same material received using the same analytical methods but in different laboratories, by various analysts, on various instruments, at different times	

TP: True positive, TN: True negative, FP: False positive, FN: False negative, PPV: Positive predictive value, NPV: Negative predictive value, LR+: Likelihood ratio for a positive test, LR-: Likelihood ratio for a negative test, ROC: Receiver-operator characteristics

the transaminases.¹⁶ A proposed approach to suspected methotrexate hepatotoxicity is outlined in Table 3.¹⁴

Take home message

Methotrexate use is associated with an increased risk of elevated transaminase levels; however, the risk of an increased risk of serious liver adverse events with modern methotrexate monitoring protocols appears to be extremely low at present. Large increases are rare, should be taken seriously and the medication stopped.

How to interpret antinuclear antibody tests

Antinuclear antibodies are autoantibodies that react with antigens in the nucleoplasm. They probably occur in the circulation of all human beings. Tests for antinuclear antibodies are only considered positive if they occur in concentration significantly above the normal serum level. Pathogenetic role is attributed to only a few antibodies. Most are thought to be caused by the disease (epiphenomena) rather than the cause of the disease (etiopathogenetic in origin).¹⁷ Antinuclear antibodies are markers for various connective tissue diseases, which have considerable overlap among each other. Very few antinuclear antibodies are specific for a single clinical entity. All connective tissue disorders are diagnosed by a set of clinical features, aided by lab tests such as antinuclear antibodies. If the clinical criteria for the diagnosis of these disorders are not fulfilled (low pre-test probability), these tests are not useful and should not be carried out.

Fluorescent antinuclear antibodies test is considered the gold standard for screening suspected cases of systemic lupus erythematosus. A positive fluorescent antinuclear antibodies test is arbitrarily defined as the level of antinuclear antibodies that exceeds the level seen in 95% of normal healthy individuals. The sensitivity of positive antinuclear antibodies test in systemic lupus erythematosus is 99%. Thus, it is a very good screening test for systemic lupus erythematosus. However, 1% of active systemic lupus erythematosus patients will show false negative result. So will the patients with isolated anti-Ro antibodies and patients with end-stage renal disease on dialysis. At the same time, many patients without any connective tissue diseases may show false positive antinuclear antibodies, e.g. those with viral infections such as HIV and hepatitis C, other autoimmune disorders such as autoimmune thyroiditis and primary biliary cholangitis (previously known as primary biliary cirrhosis), and malignancies such as lymphoma, etc.¹²

At an endpoint titre of 1:40, 32% of normal individuals are positive. At titres of 1:80, 1:160 and 1:320, the percentages of normal individuals showing positivity drop down to 13%, 5% and 3%, respectively.¹⁸ To calculate the positive predictive value of a test, we need to know the disease prevalence in the population. In other words, in a population, the prevalence of the disease is the pretest probability (probability of a person having a disease before the test result is known). For example, we know from the point prevalence data of a population survey carried out in North India that the prevalence of systemic lupus erythematosus in India is comparatively

Table 3: Management of suspected methotrexate toxicity

Transaminase monitoring	
Commencing	Every 2 weeks
Adjusting dose	Every 2 weeks
Stable dose	Every 12 weeks
Elevated transaminases	
New persistent elevation	Reduce methotrexate, investigate
New elevation >3 times upper limit normal	Withdraw methotrexate, investigate; methotrexate may be restarted after normalisation

low (3.2 per 100,000 population).¹⁹ Taking the background positivity rate of antinuclear antibodies at 1:160 as 5% and the prevalence in India roughly as 3 per 100,000, if 100,000 randomly selected Indians are tested with ANA, three persons will be true positive and 5000 will have false positive results at a titre of 1:160. Thus, the positive predictive value, Total positive/Total positive + false positive, will be 3/5003 or 0.06%. This goes on to demonstrate that, when pretest probability (in this case, prevalence of the disease in a population) is low, a positive result is more likely to be false positive. Thus, one needs to take a conservative approach when ordering antinuclear antibodies to screen for systemic lupus erythematosus in India.

However, irrespective of the population and prevalence rates, antinuclear antibodies testing is only warranted when pretest probability is high. In a retrospective study, more than 90% of patients who were referred to a tertiary rheumatology clinic for a positive antinuclear antibodies test result had no evidence for an antinuclear antibody-associated rheumatic disease. The poor predictive value of a positive antinuclear antibody in this cohort was largely attributable to unnecessary testing in patients with low pretest probabilities for antinuclear antibody-associated rheumatic disease.²⁰

Take home message

Testing for antinuclear antibodies should only be done when there is strong clinical suspicion. For any specific connective tissue disorder, screening has to be primarily done with sensitive tests and, if positive, more specific tests are to be added to the diagnostic work-up. When pretest probability is low, positive tests are likely to be false positive. Antinuclear antibodies may be present normally. Negative antinuclear antibodies may help exclude systemic lupus erythematosus. Antinuclear antibody titres do not predict disease activity. An exception is the anti-dsDNA test in systemic lupus erythematosus. Fluorescent antinuclear antibody testing should usually be ordered only once. Positive antinuclear antibody tests do not need to be repeated. Negative tests need to be repeated only if either there is a strong suspicion of an evolving connective tissue disease or a change in the patient's clinical findings suggesting that the diagnosis should be revised.²¹ The sundry autoimmunity test panels, offered by various laboratories, should be shunned as their use in the absence of clinical suspicion may cause confusion and unnecessary expenditure.¹²

Thyroid function testing: When should one test?

Thyroid-stimulating hormone is the first-line test when investigating presumed hyper- or hypothyroidism.²² However, thyroid-stimulating hormone levels exhibit diurnal variation and are affected by other medications, including steroids, opiates and some antihistamines, among others, as well as comorbidities. Chronic and acute conditions unrelated to thyroid disease can cause transient changes in

thyroid-stimulating hormone concentrations and have the potential to modify the binding capacity of plasma thyroid hormone binding proteins. Thus, thyroid-stimulating hormone should be ordered only when clinical suspicion of a thyroid problem exists.²³ The United States Preventive Services Task Force recommends against routine thyroid-stimulating hormone screening for asymptomatic adults.²⁴

For patients found to have abnormal thyroid-stimulating hormone levels, free T4 is the next test to order.²⁵ A free T4 assay is a superior indicator of thyroid status because it is not affected by changes in iodothyronine-binding proteins, which influence total hormone measurements.²³

Triiodothyronine measures can be useful in diagnosing Graves' disease, in which triiodothyronine toxicosis may be the initial symptom or an indication of a relapse. Because triiodothyronine is often a peripheral product, nonthyroid illnesses and medications can cause abnormal results as an artifact.²³

Other thyroid-specific labs include thyroid antibodies such as antithyroid peroxidase, antithyroglobulin and thyroid-stimulating hormone receptor, both blocking and stimulating. Retesting thyroid-stimulating hormone to assess treatment response should be postponed until ≥ 2 months after any change in medication or dosing.²⁶

Take home message

Thyroid studies can be very difficult to interpret. Thyroid-stimulating hormone should be the first test ordered.²⁷ However, if thyroid-stimulating hormone values do not match the clinical picture, free T4, antithyroid peroxidase antibody and other thyroid tests that are less affected by external factors can be useful.

Hemoglobin A1c and anemia

The two primary variables influencing glycosylated hemoglobin (hemoglobin A1c) are the average glucose level and the average lifespan of red blood cells.²³ Normally, there is a direct correlation between average serum glucose and hemoglobin A1c.²⁸ In patients with anemia, however, this relationship does not hold good, and may be affected by erythropoiesis and red blood cell destruction.²⁹ In iron-deficiency anemia, hemoglobin production falls secondary to iron stores, resulting in microcytic cells with a longer lifespan and elevated hemoglobin A1c.³⁰ In at least one study, haemoglobin A1c approached levels associated with diabetes (with increases as high as 1.5%) in nondiabetic patients, but resolved with iron supplementation.³¹

Increased destruction as well as increased production of red blood cells lower their lifespan and in turn decreases hemoglobin A1c levels [Table 4].²³ This can be seen in conditions such as splenomegaly and hemoglobinopathies. In patients with hemoglobinopathies, the percentage of hemoglobin A is significantly decreased, often to undetectable levels, thereby making hemoglobin A1c testing unreliable.

Take home message

In a country where diseases such as iron deficiency anemia and thalassemia are endemic in large parts, it is crucial to order for testing hemoglobin levels whenever ordering for hemoglobin A1c.

Serum ferritin: A marker of iron deficiency and an acute phase reactant

Serum ferritin is the preferred serologic marker of iron stores.³² As iron deficiency develops, low serum ferritin appears long before anemia. There are two other conditions besides iron deficiency that may be associated with low serum ferritin – hypothyroidism and scurvy. In both these situations, by the time serum ferritin falls below normal, the patient has anemia and other clinical features suggesting the diagnosis. A ferritin level less than 15 ng/mL is, thus, virtually diagnostic of iron deficiency anemia with a specificity of 99%.³³ However, a cut-off of 41 ng/mL yields sensitivity and specificity of 98% each.³⁴ Dermatologists, in India as well as elsewhere, frequently employ this test whenever a chronic diffuse telogen hair loss is suspected. This is particularly relevant in the Indian context because of the widespread prevalence of iron deficiency anemia that is one of the foremost reasons behind this kind of hair loss.³⁵ However, because low serum ferritin is a marker of subclinical anemia, a question arises regarding how to interpret low ferritin and normal hemoglobin levels. If a patient with low serum ferritin and normal hemoglobin has a low mean corpuscular volume, then (s)he has iron deficiency; if his/her mean corpuscular volume is normal, (s)he is iron-depleted. The most serious cause of the latter is gastrointestinal malignancy. Much common, and more banal, causes of iron deficiency are vegetarian diet, repeated blood donation, long-distance and competitive running and rapid rate of growth during adolescence. Interestingly, serum ferritin may be elevated in anemia of chronic disease.³⁶

Unfortunately, serum ferritin is also an acute phase reactant being elevated in chronic inflammation [Table 5].³² It has been suggested that a ferritin of less than 40 can diagnose iron deficiency anemia in patients without inflammation and less than 70 was indicative of deficiency for those with an inflammatory condition.³³

Because of such a multitude of influencing factors behind serum ferritin, the relationship of hair loss with iron deficiency (low serum ferritin) without anemia or only mild anemia is complex and controversial.³⁷ While low serum ferritin and low hemoglobin is a straightforward scenario, for which iron supplementation is recommended, a ferritin level of more than 70 ng/mL rules out iron deficiency. A level of serum ferritin between 15 and 70 ng/mL with normal hemoglobin levels is the gray zone, for which one should apply clinical judgment and delve deeper.

Take home message

Whenever ordering for serum ferritin, one must also order for the hemoglobin level and an independent acute phase reactant such as the erythrocyte sedimentation rate or c-reactive protein. In case the erythrocyte sedimentation rate/c-reactive protein is normal, 40 ng/ml should be treated as the cut-off for iron deficiency. In case the erythrocyte sedimentation rate/c-reactive protein is elevated, 70 ng/ml should be treated as the cut-off, and iron supplementation may be considered even in the absence of low Hb. Mean corpuscular volume should be the guide in such situations to assess whether the case represents iron deficiency or iron depletion.

How important is fasting in lipid profile examination

Patients are often instructed to report for fasting lab studies, specifically for lipid profiles.

Traditionally, this has been defined as an 8- to 12-hour period without

Table 4: Factors influencing hemoglobin A1c

Comorbidity	Effect on RBCs	Effect on HbA1c
Iron deficiency	RBC production decreases	Elevation
Vitamin B12 deficiency		
Lack of erythropoietin		
Pregnancy		
Renal failure		
Hemoglobinopathies	RBC destruction increases	Decline
Rheumatoid arthritis		
Splenomegaly	RBC production increases	Decline
Elevated erythropoietin		
Chronic liver disease		
Splenectomy	RBC destruction decreases	Elevation

HbA1c: Hemoglobin A1c, RBC: Red blood cell

Table 5: Factors influencing ferritin levels

Total body iron
Infection or inflammation (↑)
Female gender (↓)
Alcohol consumption (↑)
Fatty liver (↓)
Vegetarian diet (↓)
Repeated blood donation (↓)
Long-distance and competitive running (↓)
Rapid rate of growth during adolescence (↓)
Gastrointestinal malignancy (↓)
Anemia of chronic disease (↑)

↑: Elevation; ↓: Decrease

food.³⁸ Studies investigating the effect of meals on laboratory values have found that triglycerides are consistently elevated postprandially up to a maximum of 12 hours.³⁹ The effect of the fasting state on total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol is more controversial; while some postprandial differences have been detected, the clinical relevance is equivocal.⁴⁰

Nonfasting lipid values can offer useful information, particularly in patients who are unwilling or unable to return for fasting labs. The US Preventive Services Task Force supports this practice. If nonfasting lipids are used, it is crucial to factor in the postprandial effects on triglycerides and the subsequent difficulty of assessing low-density lipid cholesterol levels.²³

Take home message

The clinical relevance of postprandial vs fasting lipid levels is equivocal.

Nonfasting lipid panels have reasonable clinical utility in screening and initial treatment.⁴¹

The Road Ahead

As this editorial was getting prepared, on 15 May 2018, the World Health Organization (WHO) announced the publication of the first edition of its Model List of Essential *In Vitro* Diagnostics.⁴² In its news release, WHO cited the fact that as of today many people are unable to get tested for diseases because they cannot access diagnostic services, and many are incorrectly diagnosed.

As a result, they do not receive the treatment they need and, in some cases, may actually receive the wrong treatment.⁴² The first Essential Diagnostics List was published to address this gap that exists in getting an accurate diagnosis and an effective treatment.

The list concentrates on *in-vitro* tests, i.e. tests of human specimens such as blood and urine. It contains 113 tests, 58 of which are listed for detection and diagnosis of a wide range of common conditions, providing an essential package that can form the basis for screening and management of patients. The remaining 55 tests are designed for the detection, diagnosis and monitoring of “priority” diseases such as HIV, tuberculosis, malaria, hepatitis B and C, human papillomavirus and syphilis.⁴³ This is the first step taken on a global scale to introduce the concept of essential diagnostics, much like what its precursor, the Model List of Essential Medicines, did for the global health policy. According to the WHO, the items included in the Essential Medicines List are “drugs that satisfy the health care needs of the population [and] are intended to be available at all times.... at a price the individual and community can afford.”⁴⁴ In other words, these are the medications that can satisfy the basic health needs of a population served by physicians steeped in the principles of rational therapy. The Essential Diagnostics List seeks to extend the same principles of rationality to the field of laboratory tests.

This is a welcome move and should provide a fillip to all physicians, dermatologists included, towards rational utilization of *in vitro* tests in their diagnostic protocol.

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