Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 10 October 2022

Review Article

Factors Affecting Patients Test Results

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Received: August 29, 2022

Published: September 30, 2022

Abstract

Clinical diagnostic laboratory plays a very important role in safe delivery of quality service to the patient. Over 70 per cent of all management decisions in the clinics and hospitals are based on laboratory results. It is therefore of paramount importance that the laboratory issues out reports that are accurate, reliable and reproducible and available to clinicians in a clinically relevant time frame.

The diagnostic cycle can be divided into three phases (which has been further divided into five) with the pre analytical phase being the most error prone. A number of variables effect the results and each variable has to be controlled if we wish to obtain reliable results. Sensitivity and specificity are inherent attributes of a test, but the positive and negative predictive value depends upon the prevalence of the disease in the community. We can increase the value of the test by considering the likelihood ratio and understanding the roc of the test.

Keywords: Diagnostic; Likelihood Ratio; Sensitivity

Introduction

Diagnostic laboratories play a very important role in day to day management of patients and providing quality patient care in a clinical setting. Even though laboratory tastings are highly complex processes, it serves the backbone of any modern medicine and patient care. Health systems worldwide have increasingly dependent on reliable clinical laboratory services.

A factor that has seen to compromise laboratory medicine is the various chemicals and reagents used for measuring different analytes. Both endogenous and exogenous substances are seen to be a common challenge during test analysis. The above-mentioned substances carry out a crucial role in correct interpretation of results which is usually opposed to patient care inevitably adding to the final cost of health. At most of the times it is susceptible to errors both manual and systemic [1]. Errors that may occur will certainly lead to misleading interpretation and wrong patient management [2,3]. To assume that each variable always produces a specific effect is oversimplifying; it depends on the individual, the severity of the exposure, as well as the interval between the introduction of stress and the time of collection of the sample. It is incorrect to overstate the various factors that occur throughout the patient sample being transported to the laboratory. A thorough retrieval of the history and efficiency of the communication between the initial contact with the physician and laboratory can help minimize such factors.

Typical causes of abnormal test results besides disease

The total testing process defines the pre analytic, analytic, and post analytic phases of laboratory testing which then serves as the

core for designing and implementing interventions, restrictions, and eliminating any likelihood of errors. These errors can be categorized into 3 sections: pre-analytical, analytical, and post-analytical [4,5]. In a recent article Demissie and Musa [6] have identified factors that affect quality laboratory results which can be at levels of ordering, handling, and testing, both at preanalytical, analytical and post analytical steps. Over recent years, the ratio of eliminating error rates has incredibly decreased. recent studies demonstrate data which presents a large percentage of laboratory errors to occur in pre-analytic and post-analytic steps [7]. The first and the last categories of errors preanalytic (61.9%) and post-analytic (23.1%)) display frequent occurrences when compared to analytic errors (15%). Likewise, about one fourth of these have life threatening consequences to the patient [8].

Preanalytic errors

Steps taken at the pre-analytical stage, have been considered as major cause of errors in laboratory investigations. These may arise during patient preparation, sample collection, transportation, preparation, and storage. It is reported that the pre-analytical phase can be labeled as being error-prone. However, only recently it has been demonstrated by researchers that most errors occur in the 'pre-pre-analytical phase' that involves the healthcare personnel responsible for the initial procedures of the testing that is done outside the laboratory and not under their direct control [9].

Moreover, the patient and the specimen both are affected when pre-analytic factors are taken prior to the analyses. Furthermore, the above factors can further be divided into those acting *in vitro* (specimen handling and interference factors) and vivo (biological or physiologic).

Physiological factors

Physiologic factors are deemed beyond our control, such as age, sex, and race. These are said to be able to be managed by placing appropriate reverence limits. Moreover, characteristically categorized factors can be used to interpret test results to better understand and aid the patient. This incorporates the following elements: diet, exercise, diurnal and seasonal variations, menstrual cycle, posture and pregnancy.

The clearest effect that steers the route of each test result and its importance of stating reference intervals is age. The composition of blood in newly born infants is primarily affected by their maturity. RBC and hemoglobin values are shown to display higher levels in infants that in adults. Additionally, these gradually decrease and level out by maturity (age 15).

Following this, the adult values are then taken to be a reference for comparing the levels of young and elderly to study them in depth. A constant is the concentration levels between puberty and menopause in women and in men. Likewise, women post menopause display higher plasma concentrations of various constituents. Changes in concentration are much lower than endocrine organs response to stimuli, which concludes that hormone levels area affected by aging.

Along with the known variations of female menstrual cycles, the preovulatory increase in renin and aldosterone is also observed. Serum cholesterol levels are lower than as compared to any other menstrual cycle phase. During pregnancy, a dilutional effect can be observed in effect to the increase in the mean plasma volume, which ultimately causes hemodilution. This is then characterized by physiologic adaptations. Time plays a relatively close relationship with the fluctuations in the levels of some analytes [10].

Diurnal variations

Analytes such as cortisol, thyrotropin (TSH), growth hormone, potassium, glucose, iron, and proinflammatory cytokine exhibit diurnal variation. Analytes like cortisol, thyrotropin (TSH), potassium, glucose, and iron display diurnal variation. Hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone are released in short bursts lasting barely 2 minutes which eventually make accurate measurements impossible to obtain. Another diurnal variation are seasons. Analytes like vitamin D (which is higher during summer and lower in winters) and thyroid hormones (higher during winters and lower during summers). Altitude of measurement also has an effect of the fluctuations in the levels of the constituents in blood. Constituents like hemoglobin and hematocrit are shown to be higher at high altitudes in comparison to levels of plasma renin, transferrin, creatinine clearance, and estriol that show a decrease with increasing altitude [11].

Dietary effect

One cannot divide dietary factors in the status of the patient in the categories of "fasting" and "non-fasting." Several routine tests showed significant variation after a regular meal, indicating

that fasting time should be considered when performing tests. Triglycerides, calcium, iron, LDH, phosphorus, magnesium, lymphocytes, RBC, hemoglobin, and hematocrit are observed in the first four hours to detect any clinically significant differences. Factors like the type of diet, length of time since last meal, and test-specific dietary concerns play a steering role in the measurements taken from the patients test. Caffeine, herbal preparations, recreational drug use, ethanol, and smoking can result in both short- and long-term effects which have negative impact of the results. The level of carbohydrate and lipid metabolism differ in Africans and Caucasians, which display the steering impact race has. For instance, hormones like glucose tolerance is less in Africans, Polynesians, and native Americans in comparison to Caucasians [12].

Physical and mental stress

Stress is a situation that tend to disturb the balance between man and his environment. This heavily impacts hormone secretions and influences the concentrations of plasma constituents (TSH, glucose, insulin, aldosterone, prolactin). Moreover, stress can also effect the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis with blindness and reducing the HPA. The above must be monitored closely for the patient. There are some blind individuals whose normal diurnal variations of cortisol persist; there are others whose variations do not. As with shock and trauma, fever triggers many hormonal responses. The stress of surgery can reduce T3 (triiodothyronine) levels by 50% in patients [13,14].

Transfusions and infusions

The concentration of laboratory value can be affected by transfusions and infusions. For patients receiving an infusion, it is recommended to obtain the blood from the opposite arm. Those receiving fat emulsions must have a minimum of 8 hours before blood is drawn. An extent of hemolysis with high levels of potassium, lactate dehydrogenase (LDH), and free hemoglobin being released gradually to the age of the transfused blood can be observed in those patients who receive blood transfusions.

Exercise

Any excursion such as running up a flight of stairs or such prior to the specimen collection also affect the results obtained. A study carried out by Foren., *et al*. [15] has shown that blood levels of glucose (AST), total protein, albumin, uric acid, calcium, phosphorous, blood urea nitrogen (BUN), creatinine, total and direct bilirubin, alkaline phosphatase (ALT), and aspartate aminotransferase are elevated after short-term exercises at 4hours of a marathon. Shockingly, the next 24 hours after the race showed elevated levels of BUN, creatinine, uric acid, ALT, AST, and direct bilirubin. While all other parameters tested returned to normal.

Subjects should be guided to avoid strenuous activities prior to the day of testing to minimize pre-analytic variables that could be introduced during the blood specimen collection. Muscle damage coupled with the trauma of the surgery are seen to increase the enzyme activity in skeletal muscles which may persist for many days. At the initial stage of bed rest, plasma and extracellular volumes decrease within the early days. With a sufficient amount of bed rest, fluid retention will occur making plasma protein and albumin levels decrease by an average 0.5 and 0.3 g/dL. Incredibly, the patients posture during sample collection plays a role in affecting the concentrations of many analytes that are measured in serum or plasma. Water from intravascular to intersitial compartments can be shifted if the patients' posture is changed to an erect sitting position. This makes larger molecules not filterable. However, these effects are emphasized in patients who have a tendency for edema.

Specimen handling factors

Among controllable pre-analytic variables, specimen collection is crucial. Unacceptable specimen which is caused by misidentification, specimen quality, etc. is seen to account for the majority of preanalytical errors. Hemolysis and icteric samples are seen to have variable effects on assays which also depend upon specific testing methods and analytes**.** The variable of time and temperature for processing and storing the specimen can introduce pre-analytic variables.

Hospitals that use pneumatic tube systems of various lengths used to transport blood collection tubes to the laboratories consider and apply these variables. While reducing the pressure below the styloid pressure and also applying a tourniquet helps to maintain effective filtration pressure in the patient's capillaries. Following this step, small molecules and fluids are transported from the intranasal space to the interstitial. If the tourniquet is applied for above a minute it can result in hemoconcentration of

larger molecules which are then unable to penetrate the capillary wall. The patient should avoid extreme fist clenching during phlebotomy to further minimize pre-analytic errors.

As evidenced, medical laboratory errors may be defects within the entire process, from ordering, sample collection and transportation, and the actual testing procedures and quality control measures applied. It is also greatly influenced by patient factors as described earlier. Of all these, errors in sample collection which constitutes a major cause of concern, is avoidable. Demissie and Musa [6] in a cross-sectional study have described these issues very elaborately. In multivariate logistic regression, labeling of samples before collection, use of mixed and hemolyzed blood for testing shown by them to be factors contributing to errors.

Chemicals and salts

Heparin, ethylenediaminetetraacetic acid (EDTA), and sodium citrate are some of many widely used salts in clinical laboratories. Heparin is a commonly preferred anticoagulant for blood specimens to successfully maintain electrolyte levels. A common reason to the differences between the results of analytes in both serum and heparinized plasma is the total consumption of fibrinogen and the lysis of cellular elements that occur during clotting. The most used anticoagulant for hematologic determinations is EDTA which functions as an anticoagulant. This process is carried out by chelating calcium ions, which are required for blood clotting. Many researches discuss the use of citrate to be an anticoagulant to collect blood specimen that is primarily intended for global coagulation tests. These tests may include ones such as prothrombin time (PT) and partial thromboplastin time (PTT).

Interchanging formulations that use concentrations varying 3.2% - 3.8% in order to perform PT can negatively affect on the internationally normalized ratios (INRs). Hence it should be avoided to the best capability. Sodium fluoride and lithium iodoacetate are separately used or in combination with anticoagulants for sufficient blood collection. An hour into blood collection, a decrease or 24% of glucose levels can be seen when the inhibitors are absent in neonates. This in comparison to the 5% decrease observed in various healthy patients 'specimen which are all stored at room temperature. The anticoagulant can only have an effective morality when the collection of blood specimens to less than nominal volume increases. This then induces osmotic changes which then affects the cellular morphology. Additionally, unfractionated heparin's effective concentration can be increased beyond 14.3 U/mL causing increased binding of analytes such as calcium and magnesium to heparin. Aside from this, plasma (Lithium Heparin) has a significantly lower stability compared to serum tubes when plasma is stored after centrifugation, without being separated from the gel. Majority of the drugs seem to effect the results of both in vivo and vitro clinical laboratory tests. However, the drawback is complex, physicians have highlighted the benefits of the drug but have ignored any secondary unwanted affects. A few of the examples display steady increases in liver enzymes with dilantin and barbiturates, and increases in fibrinogen, and amylase. Access to patient history and a variety of different literatures providing similar knowledge is necessary since many medications such as anticoagulant therapies (warfarin and heparin), transfusions and blood product can provide possible replacements.

However, a subtle factor that is often overshadowed are over-the-counter drugs such as aspirin which is shown to have long lasting effects of the function of platelets. A patient's physiologic state plays a huge role in platelets function studies [16]. To gain the optimal specimen evaluation, the quality of the specimens that is submitted to laboratories is important. The technique of collection that have been established to maximize organisms and isolate pathogens should be revised prior to obtaining the specimen. Then onwards if the specimen is seen to be appropriate it can provide successful interpretation of the results. Hence why collecting specific specimens that yield pathogens should be handled. General principles should be applied but labs should also be able to improvise on specific rules for the collection of the material depending on the source of the specimen. The transportation of the specimens to the laboratories must be prompt and efficient to maximize the yield of the cultures and the analysis. Any delay can result in overgrowth or death of the microorganisms. The appropriate time for bacterial cultures lies between 2 hours of sample collection. However, if a delay is unavoidable, it is advised to be refrigerated until transported.

Analytic errors

For a long time, majority of clinical laboratories worldwide have continued their attention on methods for quality control and quality assessment programs that deal with the different factors

of testing. Two major analytical errors are instrument malfunction and operator errors. In recent years such errors have decreased to a great extent, accounting for ≤ 10% of total laboratory errors. Several different types of analytical errors leading to situations that greatly increase the variability of results have been highlighted. Preventive measures to avoid such errors have been suggested. Prevention of diagnostic errors of analytical stage begins with appropriate training of laboratory technicians and certification/ accreditation by suitable professional organizations. A well written procedure and protocol followed meticulously, avoid unnecessary analytical variations and errors [17,18]. Even though analytic problems have been reduced greatly over the previous decades, there is science that shows that interference may have serious impact on relying patients. For instance, paraproteins interfere in chemical measurements when forming precipitates during test procedures which interferes in evaluating laboratory data.

Heterophilic antibodies

These antibodies are endogenous and are found in human plasma which has the characteristic to be able to bind itself with animal antibodies and interfere immunoassays where they can bridge a gap between the capture and detection antibodies. This will result in false elevation (false positive), or rarely false depression (false negative) of measured values. False elevation may cause increase of tumor markers, endocrine tests, cardiac injury markers, and some drug levels. It may also cause false depression of serum cortisol levels resulting in wrong diagnosis of hypothalamic-pituitary-adrenal axis [19].

Interference with immunoassay systems caused by extremely high hormone levels can result in falsely analyzed data. This is synonymous to the "hook effect" which describes excess antigen concentration to result in inhibiting the immune complex. Many proteins are shown to combine with immunoglobins and high molecular weight proteins. Any clinically relevant proteins that have "macro" forms can be used to enhance the extracted results.

Immunoassay is a variable that has no time limits and is not analyte specific. It differs from patient to patients; some being lost for a long time and some for a short time. On the contrary, it does not affect all assays. Moreover, differently manufactured test kits provide differing cross reactions making the results vary lab to lad**.** Faulty results can also occur because of large numbers of sample sizes causing analytic variations. Some of these variations are: cold agglutinins, leukocytosis rouleaux, , platelet agglutination, giant platelets, lipemia, nucleated erythrocytes, megakaryocytes, red cell inclusions, cryoproteins, un-lysed erythrocytes, circulating mucin, , in vitro hemolysis, extreme microcytosis, osmotic matrix effects and bilirubinemia.

Diagnostic test values

Prior to the method being used routinely, careful protocols and evaluations must meet defined criteria. For instance, the stability and accuracy required must meet the labs patient population needs. Four commonly used indicators are used to determine the reliability of a lab test. Two of them examine accuracy and precision on how efficient different test methods perform every day in the laboratory. These two factors established and monitored by the clinical lab. Whilst the other two that examine the sensitivity and specificity, deal with how well the test can distinguish a false positive against a true positive result. The last two factors are examined by research studies done at different locations. Even though, every test has its separate set of measures and uses, laboratory tests are heavily aimed on being designed to be the most precise, accurate and sensitive as possible [20].

Accuracy and precision

The term "accuracy" (truth) refers to how well the test measures and fits the hypothesis and is defined as the percentage of correct test results (positive and negative). While a test's "precision" (repeatability) refers to how well it reproduces the same result when performed on the same patient(s). Both these concepts seem similar but are very different from one another. For instance, if a test produced the same result on 3 separate occasions, but it differed majorly from the actual referenced measurement.

"Sensitivity" can be defined as the capability of a laboratory test to successfully identify between two separate variables no matter how great the difference (those who have the disease and those who do not). On the other hand, "specificity" can be defined as the capability of a laboratory test to correctly identify those who do not have the disease. Both sensitivity and specificity are very helpful when evaluating different screening tests. Moreover, these specific test characteristics are put into being interdependent.

Figure 1: Sensitivity, specificity, and predictive values in laboratory testing. NPV, negative predictive value; PPV, positive predictive value.

Predictive values are important for assessing how useful a test will be in the clinical setting at the individual patient level. The positive predictive value (PPV) is the probability of disease in a patient with a positive test. On the other hand, a patient with a negative test result has a high negative predictive value (NPV).

A test's PPV and sensitivity are complementary when it comes to determining whether the result is a true positive. When encountered with a positive test, the PPV is displays the likelihood that the certain disease will be present in difference to sensitivity, which is the probability that the test will be positive if there the disease is present. NPV and specificity are complementary in determining true negatives. In another instance if the test result is negative, NPV is the likelihood that the disease is vague or even absent. in contrast to specificity, which is given that the disease is absent, the probability that test is negative (see Figure 1 for more information). The prevalence of a disease in a specific population determines the predictive values. A test with already mentioned sensitivity and specificity can have different predictive values in different patient populations.

Figure 2

The secondary way of measuring the accuracy of a clinical set test are called Likelihood ratios (LRs). These ratios indicate how much impact the test result will have on the odds of a disease relative to the probability of the certain disease. Each test is separated into a positive LR (PLR) and negative LR (NLR). If the test displays LR > 1 it increases the odds that the person has the specific said disease. Whereas on the other end of the spectrum, if the test displays the LR <1 it eliminates the chances of the patient carrying the disease.

Receiver operating characteristic (ROC) curves

Receiver operating characteristic (ROC) curves allow labs to identify the cutoff value that minimizes both false positives and false negatives. The plots are observed to be sensitive on the y axis while the x axis is focused on the specificity. Adding various cutoff values to the same reference population can allow to generate a curve. An optimal test would provide a cutoff value which would premise the exact split of those infected and non-infected by the disease in specific populations. This will be plotted as a right-angled curve with the fulcrum bring in the upper left corner. Moreover, as the values move from the left of the graph onwards, the sensitivity increase and the specificity decreases.

Figure 3

To compare different tests, one must calculate the are under the ROC curve. In addition, an indication of a perfectly executed test is 1. As a result, the test is better the closer the AUC is near 1. In order to find out the cutoff value for a test one should use the furthest top left corner value. This then maximizes both sensitivity and specificity as while as narrowing down any chance of false positives.

When reducing the amount of false positives and negative it is not always derived from finding the balance between sensitivity

and specificity. For instance, it may be preferable to accept the results of screening for a fatal condition that can be cured.

Postanalytic errors

Laboratory test results make up for 80% of medical records. Post-analytical mistakes depend on the establishment and design of those systems and procedures that will guarantee accurate and prompt reporting of these test findings to the patient's medical record with the correct reference range and suitable test result interpretation. It is best to avoid reporting over the phone and by hand because both methods can result in transcription errors. While the implementation of a computerized order input system in hospitals has reduced some errors, it hasn't completely eradicated the possibility of patient mismatching.

Reference intervals

In essence, the outmoded word "normal values" has been replaced with the phrase "reference values." Prior to making physiologic assessments, medical diagnoses, or treatment decisions, healthcare providers frequently compare laboratory test results to a reference interval. One can compare these in two ways: longitudinal and cross sectional. A cross-sectional comparison compares the interval of results for an analyte acquired from a group of persons who appeared to be in good health with the analyte result for a single patient. Hence being called the "population based" reference interval. A secondary scenario of the cross-sectional comparison is when a patient has their result compared to a fixed/average value. The population-based intervals can be sectioned into two main types. The most prevalent form comes from a reference group of healthy people (health associated). The second kind of reference intervals, referred to as "decision base", specifies certain medical decision thresholds that doctors utilize to make patient diagnoses or treatment decisions. Comparing a patient's most current value to earlier values for the same analyte is known as a longitudinal comparison making it easier to differences in a patient's health. Screening or diagnostic purposes require for comparisons of the patients results with the population interval. For the clinical interpretation of the laboratory test results, both healthy reference limits and disease-associated reference limits are used to interpret and evaluate lab test results. These variations are seen to be caused by the following: populations of healthy individuals, preanalytical processing procedures, and analytic platforms.

It is challenging to identify the best decision thresholds for grouping patients into "disease" and "healthy" groups. This is due to the majority of diseases have a continuum of mild and severe forms rather than being distributed uniformly. Most models fail to include the methodologic differences in laboratory test values even though many tools have been developed over the years to formalize this process. The most useful intervals are the healthy references intervals when providing a rough overview of the probability that the test value is problematic. Therefore, the guidelines for the medical decision making refer to a standard of 95%. Hence why there is less than a 1 in 20 chance for the value outside the reference interval to have matched the subject. Traditionally, the limit of acceptability is said to based upon the mean of population data because this inevitably included roughly 95% of the observations that were expected to be "normal." The best example of this is the use of multiphasic chemical profiles for screening individuals who are known to be disease-free. The chance of the presence of any disease if the screening test is irregular is as low as 0- 15%, whereas the likelihood of the test being irregular is 2-5%. Both glucose and albunime are observed to have the frequency of 5.9% and 1.5% as compared to sodium being 16.6%. when the panel had included 8 panels of tests on a multiphasic health program it displayed 25% of patients to having more than one abnormal result. Yet, when conducting 20 panel tests the value jumped to 55%.

When mentioning qualitative test reports, the cutoff value can be determined from the above given ROC curve calculations and analysis. In order to decrease any false positives or negatives, the decision limits should be moved away from the ROC optimal values. Decision limits have some limitations, while being superior instruments than reference values for determining the diagnostic value of laboratory tests.

The decision limits do not address any variation of a test result that is found to be above or below the given set limit. If the result is slightly below, it will be reported as negative and if it displays a slightly above value then it will be regarded as a positive result.

Performing the right test at the right time for the right reason

Any changes in a test result, sequential results should be taken into consideration in the context of the specific clinical setting and situation. Excessive test repetition is inefficient, and the added workload raises the risk of lab errors. The clinical state of the patient should determine the appropriate time between testing.

If s test result display negative values that does not usually eliminate any chance of some clinical diagnosis. Should the patient have no diagnosis, treatment requirement or management, the test should not be carried out. This is done to save any unnecessary health costs [20-22].

Conclusion

Preoperative prediction of postoperative complications

Various tools have been tried by many researchers in preoperatively predicting postoperative urinary retention after lumber surgery. Ken Parche., *et al.* have tried a serious of tools preoperatively in predicting common postoperative complications after lumber spine surgery. They concluded that their preoperative model can serve as a good tool for predicting postoperative complications after lumber spine surgery [23].

Similar studies also have been conducted with tools having excellent preoperative values in predicting risk factors in patients and possible complications like urinary retention following lumber spine surgery. These studies certainly help in planning lumber spine surgery in a better way in minimizing risk factors as well as postoperative complications. Also, the patients will be well aware of the possibility of such complications [24,25].

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