

Hello!

Hope you are doing well. As far as I am concerned, I went through the ordeal of primary school admission. The **amount** of preparation that children are made to do for the exam at this young age was quite **surprising but impressive**. May be it is the shape of things to come, because of adverse demand supply ratio.



There simply are not enough seats for all the interested children. So the competition is like a **baptism of fire**. **Any way let us get back to medicine This time, let us look at** - A) Widal Test B) Spurious counts on hematology analysers. C) Effect of venous stasis on hematology testing D) Using TLC as surrogate marker for CD4 counts.. So, come let us discuss these issues...

The use and abuse of Widal test...

The headline is borrowed from one of the old issues of JAPI; Many times, the Widal test is the only recourse clinician has for diagnosing typhoid. I think we all know its advantages and limitations. Despite my skepticism, this study from JOURNAL OF CLINICAL MICROBIOLOGY, says that widal test



Typhoid Mary

could be **of use for the diagnosis of typhoid fever in patients who have clinical typhoid fever** but are culture negative or in regions where bacterial culturing facilities are not available. Check out the details on **Page 2**

Spurious counts and spurious results on hematology analyzers...

Dr. Dilip brought this up in pathoindia forum and I think it is very timely. Hematology analyzers (HA) has led to a major **improvement** of cellular hematology, because of **quick and accurate results** found in most instances. **However, in several situations, spurious results are observed.**



Inadequate blood samples, situations induced by the anticoagulant(s) used, etc do **affect the results**. Check out details on **page 3**.

Venous stasis and routine hematologic testing.

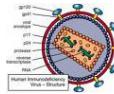
Onus to collect proper blood sample for testing is on the laboratory, in most of the settings. But, because of unorganized and complicated health scenario in India, **we don't always get the best people** for phlebotomy. So our technicians are not always infallible. This article discusses the effect of venous



stasis on some blood analytes. **All I can suggest after reading this is**, when next time you find a result that can not be explained by patient condition, **go ahead & ask** for a repeat test on a properly collected sample & see whether that solves the problem. Check out the details on **page 4**.

Total lymphocyte count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India

If I am not wrong, government has started **doing CD4 counts free of cost**, but I found that if someone at GMC knows the patient, he/she is reluctant to go there, for fear of social stigma.



In such situations if he/she can not afford the test in private labs, simple TLC could prove valuable, as this study suggests. **Details on (P. 5)**

So, this time I have tried to focus the issue on factors affecting lab tests. I hope this will prove **useful** in interpreting the tests with all their plus and minus points. **After all this is what being a part of robust, science based medical community** is about. **We don't want to do anything on intuition, or because 'we have always heard, seen or done so'**



I think this point merits some thought. **Ok, it is time to say bye; just to remind you, links to this month's interesting cases are featured on page 2. Don't forget** to check all pages and find KBC review, cricket jokes, the 'Parting thought' section, featuring Marilyn Monroe.

Thanks and regards,



~Sachin

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Issues since Feb, 2005 available online – <http://sachinkale1.tripod.com>

(1, Cont.)

Serology of Typhoid Fever in an Area of Endemicity and Its Relevance to Diagnosis



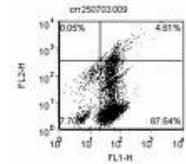
- ⌘ Currently, the **laboratory diagnosis** of typhoid fever is dependent upon either the a) **isolation** of *Salmonella enterica* subsp. *enterica* serotype Typhi from a clinical sample or b) the **detection of raised titers** of agglutinating **serum antibodies** against the lipopolysaccharide (LPS) (O) or flagellum (H) antigens of serotype Typhi (**the Widal test**).
- ⌘ In this study, the **serum antibody responses** to the LPS and flagellum antigens of serotype Typhi were investigated with individuals from a region of Vietnam in which typhoid is endemic, and their usefulness for the diagnosis of typhoid fever was evaluated.
- ⌘ The **antibody responses** to both antigens were **highly variable** among individuals infected with serotype Typhi, and **elevated antibody titers were also detected** in a high proportion of serum samples **from healthy** subjects from the community.
- ⌘ In-house enzyme-linked immunosorbent assays (ELISAs) for the detection of specific classes of anti-LPS and anti-flagellum antibodies were compared with other serologically based tests for the diagnosis of typhoid fever (Widal TO and TH, anti-serotype Typhi immunoglobulin M [IgM] dipstick, and IDEaL TUBEX).
- ⌘ At a specificity of >0.93, the sensitivities of the different tests were 0.75, 0.55, and 0.52 for the anti-LPS IgM, IgG, and IgA ELISAs, respectively; 0.28 for the anti-flagellum IgG ELISA; **0.47 and 0.32 for the Widal TO and TH tests**, respectively; and 0.77 for the anti-serotype Typhi IgM dipstick assay.
- ⌘ The **specificity of the IDEaL TUBEX was below 0.90** (sensitivity, 0.87; specificity, 0.76).
- ⌘ The serological assays based on the detection of IgM antibodies against either serotype Typhi LPS (ELISA) or **whole bacteria (dipstick) had a significantly higher sensitivity** than the Widal TO test when used with a single acute-phase serum sample ($P < 0.007$).
- ⌘ **These tests could be of use for the diagnosis of typhoid fever in patients who have clinical typhoid fever but are culture negative or in regions where bacterial culturing facilities are not available.**

Interesting Cases

Here are some cases that proved challenging to me ...

1. A 10 yr old boy presented with a 2 cm diameter swelling in sternum - since 1 year, recent increase in size; no constitutional symptoms, Tuberculin test: Negative, ESR: 14 mm (Wintrobe); CT scan impression: Tubercular osteomyelitis. FNAC impression was: non-specific inflammatory lesion ?abscess ?ostemyelitis; ZN stain: Negative for acid fast bacilli. As, positive smear of tissue for acid fast bacillus requires ~1000-10,000 mycobacteria/gm of tissue, possibility of false negative ZN stain could not be entirely excluded. With the present data, I am thinking that this is non-mycobacterial non-specific osteomyelitis. <http://telepath.patho.unibas.ch/ipath/object/view/115508>
2. 20 year old male presented with swelling in right breast, 4 cm in diameter; non-motile; firm to hard. FNAC impression "Small round cell tumor/ can not rule malignancy from breast". The young man's sister was to be married in a week. We could not give definitive diagnosis, so he was supposed to come back for excision later. But he doesn't seem to have turned up yet. Check out the patient photo, X-ray & telepathology discussion here - <http://telepath.patho.unibas.ch/ipath/object/view/113035>
3. Check out this beautiful gross image of submucous leiomyoma here – <http://www.flickr.com/photos/sachinkale/394802308/>

Spurious counts and spurious results on haematology analysers: A review



Situations leading to altered platelet counts on haematology analysers

Alteration of other parameters

Spurious decrease

- PLT agglutination (EDTA, but other anticoagulants may be concerned)
- PLT satellitism (mainly related to EDTA)
 - Around polymorphs
 - Around other WBC (normal; pathological)
- PLT-neutrophil agglutination (mainly related to EDTA)
- Large PLT (outside the normal range)
- Coagulation within the sample
- Overfilling the sample (inadequate mixing)

- PLT aggregates enumerated as WBC
- WBC count spuriously low
- Enumerated together with WBC
- Abnormal CBC
- Abnormal CBC

Spurious increase

- Fragmented RBC (schistocytes, severe iron deficiency anaemia, burns)
- Cytoplasmic fragments of nucleated cells (leukaemia, lymphoma cells)
- Cryoglobulins, cryofibrinogen
- Bacteria
- Fungi (*Candida*)
- Lipids (samples taken after a meal, lipid drips)

- RBC count spuriously low (anecdotal)
- WBC count spuriously increased
- WBC and haemoglobin spuriously high

Situations leading to altered WBC counts on haematology analysers

WBC: spurious decrease

- Agglutination of PMN (EDTA - related)
- Agglutination of WBC other than PMN (lymphocytes, lymphoma cells, leukaemic blasts)
- Excess amount of K3-EDTA anticoagulant
- Coagulation within the sample

Shrinkage of RBC: MCV and Hct↓
All parameters

WBC: spurious increase

- PLT aggregates
- (Very) large PLT
- Nucleated red blood cells
- RBC resistant to lysis (newborns, abnormal Hb, chemotherapy, uraemia, liver disease...)
- Cryoglobulin, cryofibrinogen, immunoglobulins
- Lipids
- Microorganisms (bacterial aggregates)
- Others (adipose tissue, overfilling vacuum tubes)

PLT↓
PLT↓

PLT↑
PLT↑, Hb↑, MCH↑
PLT↑
All parameters

TV under Microscope: Shahrukh's version of KBC

Wearing some else's shoes is always difficult. And if those shoes belong to big B, the difficulties are bound to be more. And this was quite evident in initial episodes of Kaun Banega Crorepati. What big B used to conduct with great dignity and little bit of formality, Shahrukh has tried convert to more informal, more youthful way. I felt that, he ended up overdoing the effort to outshine his predecessor.



But, we all know how talented and quick witted the king Khan is. And there is no better person than him, to carry forward the show, from where Amitji has left it. I am sure he will manage to make his mark.

Good thing about KBC is it tries to emphasize knowledge, and makes money a by-product of it. Important point, for someone, who would like to be a part of robust knowledge based community

(3, Cont.)

Clin Lab Haematol. 2006 Oct;28(5):332-7.

Venous stasis and routine hematologic testing.



- ⌘ Prolonged venous stasis, as generated by a long tourniquet placement, produces spurious variations in several measurable analytes.
- ⌘ To verify to what extent venous stasis influences routine hematologic testing, authors assessed routine hematologic parameters, including hemoglobin, hematocrit, red blood cell count (RBC), mean cell hemoglobin (MCH), mean cell volume (MCV), platelet count (PLT), mean platelet volume (MPV), white blood cell count (WBC) and WBC differential on the Advia 120 automated hematology analyzer in 30 healthy volunteers,
- ⌘ Either without venous stasis (no stasis) or after application of a 60 mmHg standardized external pressure by a sphygmomanometer, for 1 (1-min stasis) and 3 min (3-min stasis).
- ⌘ Although the overall correlation between measures was globally acceptable,
- ⌘ the mean values for paired samples were **significantly different** in all parameters tested, except MCV, MCH, PLT, MPV, eosinophils, basophils and large unstained cells after 1-min stasis and all parameters except MCV, MCH, MPV and basophils after 3-min venous stasis.
- ⌘ As expected RBC, hemoglobin and hematocrit displayed a **significant trend towards increase**,
- ⌘ whereas WBC and the WBC subpopulations were **decreased**.
- ⌘ Difference between measurements by Bland and Altman plots exceeded the current analytical quality specifications for desirable bias for WBC, RBC, hemoglobin, hematocrit, lymphocytes and monocytes in samples collected after either 1- and 3-min stasis. T
- ⌘ these results provide **clear evidence** that venous stasis during venipuncture might produce **spurious and clinically meaningful biases** in the measurement of several hematologic parameters,
- ⌘ prompting further considerations on the usefulness of **adopting appropriate preventive measures** for minimizing such influences.

PJs: Cricket jokes

Indian Team Manager : "Hello" (over Phone)
Caller : "Can I talk to dada Please, I am his friend and calling from Hyderabad."
Indian Team Manager: "Sorry, he went to bat"
Dada's friend: "No Problem Manager, I will Hold on"



Ajit: Maikal, Test Match mein kyaa ho raha hai ?
Maikal: Boss, Vivian Richards chhakke pe chhakke maar raha hai.
Ajit: Saaleh ko sabak sikhana padega. Lunch break mein usse phone milana.
Maikal: Yes Boss.
AJIT: (on phone, to Richards): Veeveeyun Reechards, tumhari Maa hamare kabze mein hai!

(4, Cont.)

J Acquir Immune Defic Syndr. 2002 Dec 1;31(4):378-83.

Total lymphocyte count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India and other resource - constrained countries.



- ⌘ In most resource-constrained countries, CD4 cell count testing is prohibitively expensive for routine clinical use and is not widely available.
- ⌘ As a result, physicians are often required to make decisions about opportunistic infection (OI) chemoprophylaxis without a laboratory evaluation of HIV stage and level of immunosuppression.
- ⌘ Objective of this study was to evaluate the correlation of total lymphocyte count (TLC), an inexpensive and widely available parameter, to CD4 count.
- ⌘ To determine a range of TLC cutoffs for the initiation of OI prophylaxis that is appropriate for resource-constrained settings.
- ⌘ Spearman correlation between CD4 count and TLC was assessed in patients attending an HIV/AIDS clinic in South India.
- ⌘ Positive predictive value (PPV), negative predictive value (NPV), and sensitivity and specificity of various TLC cutoffs were computed for CD4 count <200 cells/mm³ and <350 cells/mm³.
- ⌘ Correlation and statistical indices computed for all patients and for patients dually infected with HIV and active tuberculosis.
- ⌘ High degree of correlation was noted between 650 paired CD4 and TLC counts ($r = 0.744$).
- ⌘ TLC <1400 cells/mm³ had a 76% PPV, 86% NPV, and was 73% sensitive, 88% specific for CD4 count <200 cells/mm³.
- ⌘ TLC <1700 cells/mm³ had a 86% PPV, 69% NPV, and was 70% sensitive, 86% specific for CD4 count <350 cells/mm³.
- ⌘ The cost of a single CD4 count in India is approximately 30 US dollars, whereas the cost of a single TLC is 0.80 US dollars.
- ⌘ **CONCLUSION: TLC could serve as a low-cost tool for determining both a patient's risk of OI and when to initiate prophylaxis in resource-constrained settings.**
- ⌘ **PPV, NPV, sensitivity, and specificity maximally aggregated at TLC <1400 cells/mm³ for CD4 <200 cell/mm³ and TLC <1700 cells/mm³ for CD4 <350 cells/mm³.**
- ⌘ Selection of appropriate TLC cutoffs for prophylaxis administration should be made on a regional basis depending on OI incidence, antimicrobial resistance patterns, and availability of the antimicrobials.

Parting Thought...



"A career is a wonderful thing, but you can't snuggle up to it on a cold night."

~ Marilyn Monroe

An American Golden Globe Award winning actress, singer, model and pop icon.

(End, 5)