

Potential hematological biosignatures as screening tools for tuberculosis co- infection among people living with HIV

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Abstract

Background: A routine laboratory test like complete blood count is a common practise during any hospital visit. It doesn't provoke social stigma, especially when diagnosis of infectious diseases like tuberculosis and Human immuno deficiency virus (HIV) infection are to be done. Thus if tested for its potential to diagnose or screen tuberculosis co-infection among people living with HIV, it will serve as a valuable laboratory evidence to start treatment in such vulnerable population. We hereby address the plausibility of using haematological parameters as screening tools to identify TB among PLHIV.

Methods: Our retrospective analysis included four cohorts of people - 259 healthy volunteers, 299 newly diagnosed tuberculosis patients, 135 PLHIV and 255 HIV-TB co-infected patients, wherein we tried to identify haematological variables with higher potential to distinguish among these groups.

Results: We report for the first time, a three analyte haematological signature (anemic- decreased CD4- increased MLR) which has higher capacity to serve as HIV-TB screening tool among PLHIV population (sensitivity-88.9%, specificity-91.7%, AUC-0.965). Another three analyte signature comprising increased MLR- Neutrophilic- Thrombocytic- nature has a sensitivity of 90.1 %, specificity of 91.3 % and AUC 0.957 in differentiating healthy people from pulmonary tuberculosis patients. Followed by this, Lymphocyte percentage and Monocyte Lymphocyte Ratio as single haematological markers have a sensitivity, specificity, AUC of 90.2%, 83.6%, 0.92 and 88.3%, 88 %, 0.931 respectively in differentiating healthy from pulmonary tuberculosis population.

Conclusion: Further studies supporting this three analyte panel as biosignature for easy and effective screening of HIV-TB among the vulnerable PLHIV cohort is warranted, which is the need of the hour to prevent mortality caused by this co-infection.

Clinical trial registration: Two studies were registered with the Clinical Trial Registry (CTRI 2008/091/000024 & NCT00933790).

BACKGROUND

An estimated 10.6 million people (95% uncertainty interval: 9.9-11 million) fell ill with tuberculosis (TB) in 2021 [1]. People living with human immuno deficiency virus (PLHIV) are more likely than others to become infected with TB [2]. For patients co-infected with HIV and TB, both anti tuberculosis therapy (ATT) and anti-retroviral therapy (ART) are required to prevent deaths occurring due to either of these two diseases. Providing prompt TB treatment and early initiation of ART to PLHIV diagnosed with TB, has averted 74 million deaths between 2000 and 2021 [1]. The provision of ART for PLHIV among notified newly diagnosed TB has been 89% since 2019. However, when compared to the total number of PLHIV estimated to have developed TB in 2021, the coverage reduced to a mere 46%, far below the overall level of coverage of ART for PLHIV, which was 75% [3]. The primary reason for this difference is the big gap between the estimated (703,000) and reported (368,641) number of PLHIV who developed TB in 2021 [1]. In high burden countries like India where the estimated numbers are far higher than the actually diagnosed TB cases, mortality prediction using routine clinical laboratory assays rather than any other confirmatory testing looks lucrative. Although the tests are non-definitive, these tests could still assist TB diagnosis and prognosis at a low cost.

Inflammation in TB is very critical; many inflammatory cells including macrophages, monocytes, neutrophils, primed T cells and B cells are recruited to the site of infection. Following recruitment, several pro- and anti-inflammatory cytokines, chemokines and proteins are produced by these cells in an attempt to control the infection [4]. Besides immune cells, platelet activity is also documented to increase in pulmonary TB patients as compared to healthy volunteers [5] and this has been correlated with severity of TB disease [6]. A high neutrophil count has been claimed to be a useful predictor of unfavourable TB treatment outcome [7], with TB causing alterations in the haematological profiles as well.

Abnormalities in blood profiles are well documented among HIV-infected persons. Anemia is reported to be associated with disease advancement and increase in mortality [8, 9]. Thrombocytopenia, a condition with decreased platelet count is

prevalent in the later stages of disease where the CD4 cell count touches abysmal levels [10]. Leukopenia or decrease in white blood cells is also a common accompaniment especially in advanced HIV disease [11]. Even though it is well established that both HIV and TB cause haematological aberrations, very few studies have explored into the haematological profiles or complete blood count (CBC) of pulmonary tuberculosis (PTB) and PLHIV with a view to understand their importance as screening tools to identify HIV-TB co-infection. Few studies that have documented altered hematological profile in co-infected participants, including a study from Northwest Ethiopia which demonstrated higher levels of neutropenia, anemia, and thrombocytopenia among HIV-TB co-infected patients compared to TB group [12]. Another study from Guyana reported decreased haemoglobin and WBC counts in the co-infected cohort compared to PTB group [13]. Haematological abnormalities worsen in HIV-TB coinfection has been ascertained in both the studies. In a high burden setting, as HIV infection increases the risk of getting TB disease [14], we were interested to evaluate the use of haematological parameters in determining the possibility of detecting TB in PLHIV participants and vice versa. Although there are existing tuberculosis screening tests or tools recommended by WHO in PLHIV like symptom screening, CRP, urine LAM, etc, a routine haematology test has an advantage over these specific confirmatory tests wherein it doesn't require additional tests and does not provoke social stigma, especially in diseases like TB and HIV. Hence we analysed haematological parameters from four different cohorts of people to identify their significance as screening indices for HIV-TB coinfection among PLHIV.

METHODS

Study population and data collection

This retrospective analysis involved four different group of people namely healthy volunteers (HV), PLHIV, PTB and HIV-TB who were recruited from different parts of Tamil Nadu. Their haematological values were compared to understand the possibility of using these parameters in differentiating one group from the other. The data were extracted from randomised clinical trials conducted at ICMR- National Institute for Research in Tuberculosis in the past 10 years. Participants included adults aged 18-65 years. Moribund patients, pregnant and lactating women were excluded in the trials. The PLHIV group consisted of newly diagnosed HIV ART-naïve patients. The PTB group included newly diagnosed smear positive cases before the start of ATT. The HIV-TB group comprised of PLHIV who were newly diagnosed with PTB started on ART as per prevailing guidelines.

Measurement of Haematological Markers

Briefly, in all the studies, 2 ml of EDTA blood was collected from participants and processed in Ac•T 5diff CP (Cap Pierce) Haematology Analyser (Beckman Coulter) and the results were documented.

Approvals

All trials were conducted with prior approval of the institutional ethical committee and after obtaining informed written consent. Except for the HIV-TB and PLHIV groups, all other participants were HIV negative. These cohorts who had concomitant TB had culture confirmed, rifampicin sensitive pulmonary TB and were not suffering from any other opportunistic infection.

Statistical Analysis

For this analysis, data was extracted from records, checked for completeness and entered into IBM SPSS Statistics applicable to Windows (IBM Corp. Released 2017, Version 25.0. Armonk, NY). Data were analysed using Mann-Whitney U test for comparison between two groups and analysis of variance (ANOVA) for comparison between more than two groups. Receiver operating characteristic (ROC) curve analysis was performed to determine the discriminatory power of the hematological parameters ie. sensitivity and specificity of individual haematological candidate markers to distinguish

between the different study groups. Combinations of haematological parameters were analysed through integrative ROC using the freely available web application CombiROC v.1.2.^[17]

Outcome Definitions

Anaemia was defined as Hgb < 12.3 g/dl for males and Hgb < 9.9 g/dl for females [15]. Lymphocytopenia, neutropenia and thrombocytopenia were defined as lymphocytes < 18%, platelets < 130×10^3 cells/ml and neutrophils < 44% & < 42% in females and males respectively. On the other hand, lymphocytosis, neutrophilia and thrombocytosis are defined as lymphocytes > 45%, neutrophils > 75% & > 74% and platelets > 420×10^3 cells/ml & > 380×10^3 cells/ml in females and males respectively [15].

RESULTS

A total of 948 participants comprising 135 PLHIV, 259 HV, 255 HIV-TB and 299 PTB patients were included in this analysis. The various haematological parameters studied and their statistical significance among the four study groups are documented in Table 1.

Haematological abnormalities in the study groups

Using the haematological observations, the prevalence of various haematological abnormalities were analysed and their values are given in Table 2. We found that 12% of healthy controls and 43% of TB group had anaemia. The PLHIV population showed only 13% prevalence of anaemia, however upon coinfection with TB, it increased to 84%. Similarly, thrombocytosis (30%) and neutrophilia (26%) were also highly prevalent among HIV-TB cases, whereas their prevalence rates were only 5% and 3% in the PLHIV group. The PTB group had highest prevalence of thrombocytosis (57%) and neutrophilia (27%). We also observed a higher prevalence of lymphocytopenia in the TB (56%) and HIV-TB (38%) groups compared to the PLHIV group (4%). Thus our observations suggest that haematological abnormalities increase manifold during HIV-TB coinfection compared to PLHIV condition.

ROC analysis reveals the best haematological biomarker as lymphocyte percentage to differentiate TB from healthy controls

The ability of haematological parameters to differentiate between the HV, PLHIV, PTB and HIV-TB groups was analysed by determining the sensitivity, specificity and area under curve (AUC) (Table 3). Since, neutrophil lymphocyte ratio (NLR) and monocyte lymphocyte ratio (MLR) have gained importance as predictors of various pathologies, we also calculated NLR and MLR and compared them between the groups. As single analytes, lymphocyte percentage gave a sensitivity of 90.2% and specificity of 83.6% (AUC-0.92) and MLR gave a sensitivity of 88.3% and specificity of 88% (AUC- 0.931) in differentiating healthy from TB group. This was followed by NLR, with a sensitivity of 87.0% and specificity of 84.1% (AUC-0.90).

Three analyte signature differentiate PTB from HIV-TB

Since, other haematological parameters as individual components had lower AUC in differentiating between other groups, in order to increase their target product profile (TPP) for diagnostic markers according to World Health Organisation (WHO) [16], we tested combinations of haematological parameters to determine their diagnostic / screening potential. This was achieved with the help of CombiROC software [17] which computes sensitivity and specificity for different marker combinations and performs a combined ROC analysis. This analysis gave better sensitivity, specificity and AUC for certain analytes and the best combination of haematological markers and their sensitivity, specificity and AUC are shown in the provided Figures 1 and 2. Using this approach, we hereby report a three signature panel of haematological parameters which hold promise for distinguishing HIV-TB from PLHIV with AUC, sensitivity and specificity meeting the TPP standards of WHO required for a good diagnostic biomarker. As seen in Figure 1, an anaemic- decreased CD4 - increased MLR signature was associated with HIV-TB co-infection with a sensitivity of 88.9% and specificity of 91.7% compared to HIV infection alone. We also found that a three signature haematology panel, comprising of increased MLR- thrombocytotic- neutrophilic nature, which gave a sensitivity of

90.1% and specificity of 91.3% in differentiating healthy from TB group (Figure 2) suggesting that, this three analyte panel has the potential to serve as a screening tool for TB among general population.

DISCUSSION

As WHO recommends TB screening for all PLHIV before the initiation of ART [18] and haematological profile gives a snapshot of the body's functionality, we questioned the possibility of using haematological parameters for this purpose. In order to identify differences in various haematological parameters and their ability to distinguish between individuals with PTB, PLHIV and HIV-TB coinfection from healthy controls, we analysed the haematological. Since the sensitivity of sputum microscopy falls within 24-61% [19] in PLHIV population, we ventured in with this novel approach of using the hemogram analysis which is a routine test done during any hospital visit, both in the outpatient and inpatient departments in order to evaluate its usefulness not only in TB diagnosis in the immunocompromised but also treaded one step ahead to see if it could distinguish the sub-groups as well. Haematology, if proven to be a good diagnostic/ screening tool may aid in early detection of HIV-TB coinfection, which will greatly benefit the society. Variations in haematological profile is a common phenomenon during any infection/ disease [20]. These changes are well documented across different infectious diseases including TB [21] and HIV [22].

As expected, the haematological parameters in healthy controls differed from the rest of three study groups. Of particular interest, as reported earlier [23, 24] we observed increased prevalence of anaemia among PTB participants and the prevalence of anaemia was further exacerbated by the HIV coinfection. This emphasises the fact that anaemia seen in PTB is due to the chest inflammatory conditions and not nutritional anaemia always. Thus, there is a possibility that control of PTB can reverse the anaemia status. This observation also reinforces the fact that special care should be given to TB and HIV-TB people with respect to redeeming their haemoglobin status to normal values for a successful recovery. Similarly, the increased prevalence of neutrophilia and lymphocytopenia in those with HIV-TB coinfection as compared to HIV infection alone, raises our opinion that these cells not only in their functions, but also in their numbers respond uniquely to infections.

In recent times, rather than the hematological analytes by themselves, their ratios have gained importance in various diagnoses. NLR has gained prominence as a prognostic/ diagnostic marker for cardiovascular and inflammatory diseases, coronavirus disease and several types of cancer [25-28] as well as respiratory disease like pneumonia and different forms of TB [29, 30]. Analogous to the above reports, we also found that NLR has great potential to serve as a screening tool to aid in TB diagnosis with a sensitivity of 87%. In addition to NLR, our analysis confirms the fact that MLR can be considered as a crucial biomarker to identify TB in adults (31).

Even though most of the parameters analysed were statistically different between the study groups, their diagnosing accuracy in differentiating between the groups was not satisfactory. Area under the curve was inadequate to rank them as good diagnostic markers as per the WHO's, TPP for a diagnostic biomarker for tuberculosis with or without HIV. However, lymphocyte numbers (Table 3) had the greatest discriminative power compared to all other tested haematological parameters, in distinguishing TB from the healthy group. We did a combinatorial analysis of haematological parameters to check their diagnostic ability in differentiating between the four groups. By this method we report two different three signature panels, one for differentiating TB from healthy group and one for differentiating HIV-TB from PLHIV.

Even though it is widely agreed that these parameters are shown to be influenced during co-infections like PTB and HIV [12, 13] to our knowledge, no study has documented the use of these parameters as a screening criteria in the dually infected. With our observations presented here we provide small leads towards using haematological parameters as screening tools for HIV-TB coinfection among PLHIV group, in a low resource setting. Even though our data are from well characterized dataset, interpretation of haematological data should be made keeping in mind, underlying confounding factors like intake of drugs, diabetes, age and so forth. Also, the range identified here for the biosignatures cannot be generalized for diverse population across the globe. Further validation using multi-centric and multi-national cohorts will add strength to our investigations. Additional biomarkers like C- reactive protein might be added in the future studies, which is a limitation of our analysis. Presumptive TB cases identified by these signatures, may be subjected to further confirmatory tests like Xpert,

culture and chest x-ray which will help in early diagnosis of TB among HIV positive individuals and help in rapid ATT initiation among this vulnerable population.

CONCLUSIONS

Haematological parameters are indicative of prevailing infections and are cost effective and easily accessible. Our findings reveal that a combination of haematological parameters can be used as screening aid for HIV-TB coinfection. This finding has to be validated in a large multi-centric study before further implementation. Haematological screening will reduce the stigma in patients being asked to get tested for TB, thus increasing TB screening, rapid detection and early initiation of treatment, thereby contributing to TB control in the long run.

Abbreviations

TB: Tuberculosis

PLHIV: People living with human immuno deficiency virus infection

ATT: Anti tuberculosis therapy

ART: Anti-retroviral therapy

ANOVA: Analysis of variance

ROC: Receiver operating characteristic

AUC: Area under curve

NLR: Neutrophil lymphocyte ratio

WHO: World health organization

Hgb: Hemoglobin

Declarations

Ethics approval statement: All the studies from which data has been obtained were done with prior approval NIRT- IEC (TRC- IEC No: 2008002/ NIRT IEC No: 2011001/ NIRT IEC No: 2015023).

Availability of data: De- identified data may be shared with qualified researchers upon request to the corresponding author.

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Competing Interests: No financial & non-financial competing interests to declare.

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Tables

Table 1: Comparison of hematological profile among different study groups

Characteristic	All	PLHIV (G1)	HV (G2)	HIV-TB (G3)	TB (G4)	P value	(Groups with P value <0.05)
	(n=948)	(n=135)	(n=259)	(n=255)	(n=299)		
M: F	625:323	66:99	134:125	196:59	229:70		
Age (years) #	40.0± 12.3	38.3± 8.5	47.0±14.0	39.1±8.8	35.3± 12.2		
Hgb (g/dl) #	11.7± 2.5	13.1± 2.1	12.9±2.1	9.7±2.1	11.9± 2.2	<0.001	(G2 and G4; G2 and G3; G1 and G3; G1 and G4; G3 and G4)
RBC (10 ⁶ /μL) #	4.3±0.9	4.3±0.7	4.7±1.1	3.6±0.8	4.5±0.6	<0.001	(All groups)
WBC #	8067.8±2922.8	6722.3±2173.0	8086.6± 2539.2	6938.2± 3148.4	9622.4±2580.0	<0.001	(G2 and G4; G2 and G3; G1 and G2; G1 and G4; G3 and G4)
Platelet # (x10 ³ /μL)	3.3±1.3	2.5±1.0	2.8±0.9	3.4±1.4	4.1±1.3	<0.001	(All groups)
PCV	35.6± 18.3	38.3± 5.6	39.2± 26.7	30.7± 16.2	35.6± 13.4	<0.001	(G2 and G4; G2 and G3; G1 and G3; G1 and G4; G3 and G4)
Neutrophil (%) #	62.1±11.8	51.7± 10.7	57.2±8.1	65.0±13.4	68.5± 7.9	<0.001	(All groups)
Lymphocytes (%) #	25.1± 12.6	34.9± 8.6	31.2±7.4	22.1±10.1	18.1± 14.2	<0.001	(All groups)
Eosinophil (%) #	4.2±3.9	5.5±5.3	4.6±3.3	3.0±3.1	4.3±4.1	<0.001	(G2 and G3; G1 and G2; G1 and G3; G1 and G4; G3 and G4)
Monocytes (%) #	8.2±3.4	7.3±2.6	6.3±2.5	9.2±4.5	9.3±2.5	<0.001	(G2 and G4; G2 and G3; G1 and G2; G1 and G3; G1 and G4)
Basophil (%) #	0.6±0.5	0.5±0.5	0.7±0.4	0.6±0.5	0.6±0.7	0.006	(G2 and G3; G1 and G2)
CD4 #	316.0± 272.3	525.3± 272.0		204.7± 196.8		<0.001	(G1 and G3)

CD8 #	910.7±558.8	1160.5±520.3		777.9± 533.3		<0.001	(G1 and G3)
CD4/CD8 #	0.4±0.3	0.6±0.4		0.3±0.3		<0.001	(G1 and G3)
NLR #	3.4±3.1	1.7±1.0	2.1±1.1	4.3±4.8	4.6±2.2	<0.001	(G2 and G4; G2 and G3; G1 and G2; G1 and G3; G1 and G4)
MLR #	0.42±0.32	0.22±0.09	0.23±0.14	0.51±0.42	0.60±0.26	<0.001	(G1 and G3; G1 and G4; G3 and G4; G2 and G4; G2 and G3)

Plus-Minus values are mean ± SD;

Hgb - Haemoglobin ; RBC- Red Blood Cell; WBC- White Blood Cell; NLR- Neutrophil Lymphocyte Ratio; n- number, P≤0.05 is considered statistically significant.

Table 2: Prevalence of haematological abnormalities among different study groups

	Healthy (N=259)	TB (N=299)	PLHIV (N=135)	HIV-TB (N=255)
Neutropenia (F<44%, M<42%)	9	1	29	15
Neutrophilia (F>75%, M>74%)*	7 (3)	71 (24)	4 (3)	67 (26)
Neutrophils within range	243	227	102	173
Thrombocytopenia (<130 x103 platelets)	10	5	6	18
Thrombocytosis (F>420 x103 platelets, M>380 x103 platelets)*	17 (7)	171 (57)	7 (5)	76 (30)
Platelets within range	232	123	122	161
Anemia (<12.3g/dl in men, <9.9g/dl in women)*	32 (12)	130 (43)	18 (13)	214 (84)
HgB within range	227	169	117	41
Lymphocytopenia (<18%)*	14 (5)	167 (56)	5 (4)	98 (38)
Lymphocytosis (>45%)	7	1	17	6
Lymphocyte count within range	238	131	113	151

*N (%) – number (percentage); F- female, M- male

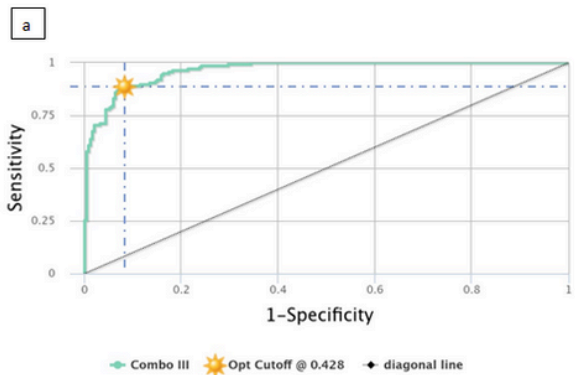
Table 3: Sensitivity and specificity of haematological parameters as determined by ROC in differentiating the four study groups

Variables	Healthy vs TB			Healthy vs HIV-TB			TB vs HIV-TB			PLHIV vs HIV-TB		
	Se%	Sp%	AUC	Se%	Sp%	AUC	Se%	Sp%	AUC	Se%	Sp%	AUC
WBC	69.02	61.89	0.690	36.76	92.21	0.620	89.56	54.55	0.749	78.26	90.22	0.516
RBC	65.32	50.41	0.580	77.87	82.38	0.841	86.53	67.19	0.820	69.17	72.93	0.753
N%	79.12	79.10	0.843	64.03	72.28	0.72	87.83	30.43	0.570	64.82	85.71	0.800
L%	90.24	83.61	0.922	64.03	84.43	0.773	87.54	38.74	0.633	71.54	85.71	0.829
M%	80.47	73.77	0.834	62.45	78.28	0.74	82.49	33.60	0.550	51.68	71.43	0.647
B%	69.02	51.64	0.603	73.91	51.64	0.652	53.20	60.08	0.553	77.47	43.61	0.559
E%	32.32	85.66	0.576	51.38	85.66	0.720	61.95	60.47	0.644	66.80	69.92	0.723
Platelet	64.98	86.48	0.820	44.66	81.56	0.632	63.64	64.43	0.67	68.77	65.41	0.695
Hgb	82.49	38.11	0.639	69.57	87.70	0.863	78.79	64.03	0.780	84.58	77.44	0.877
CD4	-	-	-	-	-	-	-	-	-	77.08	86.47	0.865
CD8	-	-	-	-	-	-	-	-	-	50.20	84.21	0.720
CD4/CD8	-	-	-	-	-	-	-	-	-	69.17	68.42	0.739
NLR	87.0	84.1	0.906	66.54	80.31	0.753	41.73	84.62	0.618	66.9	87.4	0.821
MLR	88.3	88	0.931	67.8	86.1	0.808	47.8	79.6	0.633	68.6	88.9	0.811

Units; % -percentages; Se% - sensitivity, Sp% - specificity, AUC – area under the curve

Figures

Figure 1 PLHIV Vs HIV-TB



Parameters	AUC	SE	SP	OPT CUT OFF
Hgb- CD4 count-MLR	0.965	0.889	0.917	0.428



Parameters	False Negative	False Positive	True Negative	True Positive	CLASS A (PLHIV)	CLASS B (HIV-TB)
Hgb- CD4 count-MLR	3.9	5.4	59.8	30.9	34.8	65.2

Figure 1

a) The identified 3 signature panel comprising Hgb- CD4 count- MLR is able to distinguish between PLHIV and HIV-TB group with a sensitivity of 88.9%, specificity of 91.7% and AUC of 0.965 b) The predictive value of true negatives (TN) is 59.8 %, true positives (TP) is 30.9 %, false negatives (FN) is 3.9% and false positives (FP) is 5.4 % by this signature panel. CombiROC application was used to identify the optimal combinations.

Figure 2- Healthy Vs TB

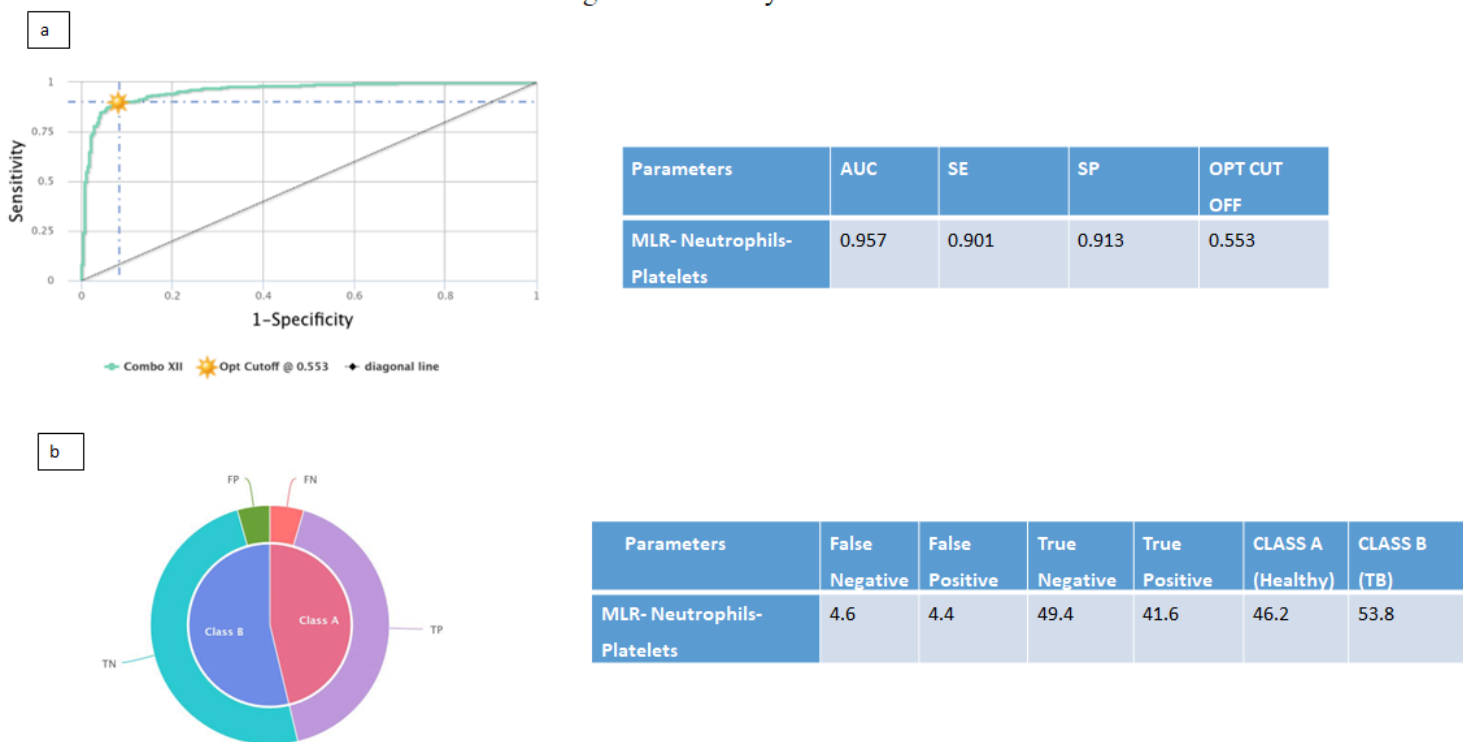


Figure 2

a) The identified 3 signature panel comprising MLR- Platelet count- Neutrophil % is able to distinguish between healthy and TB group with a sensitivity of 90.1%, specificity of 91.3% and AUC of 0.95. b) The predictive value of true negatives (TN) is 49.4 %, true positives (TP) is 41.6 %, false negatives (FN) is 4.6% and false positives (FP) is 4.4% by this signature panel. CombiROC application was used to identify the optimal combinations.