Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/pp91ap05

URINARY NEOPTERIN A SURROGATE MARKER OF ACTIVE TUBERCULOSIS AND ASSESMENT OF TREATMENT OUTCOME

Dr. Alok Chandra[1], Dr. Bal Krishna Kushwaha[2*], Dr. Shveta Sachdeva[3], Dr. Saumya Singh[4]

[1]Professor & Head, Department of Respiratory Medicine, United Institute of Medical Science, Prayagraj, Uttar Pradesh, India
[2*]Assistant Professor, Department of Respiratory Medicine, United Institute of Medical Science, Prayagraj, Uttar Pradesh, India
[3]Assistant Professor, Department of Pathology, Motil Lal Nehru Medical College, Prayagraj, Uttar Pradesh
[4]Professor & Head, Department of Microbiology, United Institute of Medical Science, Prayagraj, Uttar Pradesh, India

***Corresponding Author:** Dr Bal Krishna Kushwaha
*Respiratory Medicine, United Institute of Medical Science, Prayagraj, Uttar Pradesh, India
Email: drbkpulmonary2014@gmail.com

Abstract-Background- There are several advancements in the diagnostic tests of tuberculosis but obtaining material from nonproductive pulmonary as well as extrapulmonary cases still remains a challenge. This prompted us to search for a non invasive marker to diagnose active TB.
Methods- Total 100 subjects were enrolled in study. Categorization of patients into active, LTBI and control done with the help of Mantoux test, IGRA, smear microscopy and CBNAAT. 10 to 15 ml spot urine sample from all the subjects was collected in sterile container and sent to pathology lab for the estimation of neopterin level in urine by ELISA method determined by optical density. Patients having active disease antitubercular treatment started after doing baseline urinary nepoterin test and follow up test was also done at the end of 3 month and 6 month (end of treatment).
Results- Our study compare the urinary neopterin value between active TB, latent TB and normal subjects. It was found that there was statistically significant difference in the base line urinary neopterin level among active cases , LTBI and control. Among active TB cases after the initiation of standard first line anti tubercular treatment follow up urinary neopterin test was also done at 3 months and at 6 months. Comparing urinary neopterin from base line to 3 month and at 6 month it was found statistically significant (p value<0.05). Further more on comparison of urinary neopterine at 3 month and 6 month it was statistically insignificant (p value >0.05).
Conclusion-Urinary neopterin can be considered as a surrogate marker to diagnose active TB and treatment outcome.

Introduction

Tuberculosis is a common disease worldwide but there is no specific marker to differentiate active from latent tuberculosis. Biomarkers to distinguish latent from active tuberculosis is lacking in clinical practice. Early suspicion and diagnosis of active disease and early start of treatment are essential and of paramount importance to prevent transmission and containment of disease and so to curtail MDR and XDR cases. X ray chest PA view coupled with sputum microscopy and culture

really confirms productive pulmonary disease but not so in cases who do not expectorate. Also can not differentiate between latent and active pulmonary and extra pulmonary disease unless disease tissue is available which is mostly an invasive procedure .As per the 2010 report of RNTCP of India average number of notified extra pulmonary TB cases was 26% which increased to 40% by 2021.For elimination of TB under NTEP we will have to pay more attention on the diagnosis of EPTB as well. This prompted us to search for a non-invasive surrogate marker to diagnose active TB.

Scientists found a molecule neopterin produced by T lymphocyte can differentiate active from latent TB [1,2]. Neopterin in chemical nomenclature 2-amino-4hydroxy-6-(D-erythro-1,2,3-trihydroxypropyl)-pteridine produced by activated monocytes, macrophages, dendritic cells, and endothelial cells and to a lesser extent by renal epithelial cells, fibroblasts, and vascular smooth muscle cells upon stimulation mainly by interferon-gamma and to a lesser extent by interferon alpha and beta with its release being enhanced by tumor necrosis factor [3]. Neopterin is, after production, secreted unaltered in urine. Urinary neopterin levels can be determined by a simple ELISA system [4]. A previous study reported urinary neopterin/creatinine ratio in patients with active tuberculosis and found them to be higher than in patients with pneumonia and lung cancer [5].

Material and methods

This is an observational study. Total 100 subjects aged between 18 and 50 year were enrolled in study. 52 subjects having clinical suspicion of active pulmonary or extra pulmonary TB and positive on smear microscopy or CBNAAT were labelled as active disease . 24 subjects were taken from the contact of active cases who had neither clinical nor radiological evidence of activity but gave positive IGRA or mantoux test were labelled as Latent TB. 24 healthy relatives testing negative with Mantoux and IGRA were included as controls. 10 to 15 ml spot urine sample from all the subjects was collected in sterile container and sent to pathology lab sun protected for the estimation of neopterin level in urine by ELISA method determined by optical density with Erba ELISA LISA SCANEN machine and reports were given in 1 hr. Patients having active disease antitubercular treatment started after doing baseline urinary nepoterin test and follow up test was also done at the end of 3 and 6 months (end of treatment). Follow up tests were not done in case of latent TB and normal subjects.

Subjects having chronic renal disease, occupational lung disease, malignancy and other chronic illnesses were excluded from study. Previously treated patients and patients who were already on anti tubercular treatment at the time of enrollment were also not included.

Results - In our study the mean age of the patient was 26.2 year, males were more as compared to females. Maximum number of cases were having pulmonary tuberculosis(57.96%). Among extrapulmonary tuberculosis maximum cases are of pleural effusion (23.07%) followed by lymph node tuberculosis(11.53%). Mean Urinary neopterin value among active TB, latent TB and normal subjects was 5.67, 2.23 and 1.23 respectively. It was found that there was statistically significant difference in the base line urinary neopterin level among active cases (mean 5.67), LTBI (mean 2.23) and control (mean 1.23). Among active TB cases after the initiation of anti tubercular treatment (HRZE - HRE) follow up urinary neopterin test also done at 3 month and at 6 month. It was found that comparing urinary neopterin from base line to 3 month and at 6 month it was statistically significant (p value<0.05). further more on comparison of urinary neopterine at 3 month and 6 month it was statistically insignificant (p value >0.05) using ANOVA post hoc analysis.

Table: 01: Distribution of cases of tuberculosis patients:

Tuberculosis	**Frequency**	**Percentage**
Pulmonary Tuberculosis	30	57.69%
Lymph node tuberculosis	6	11.53%
Endometrial	2	3.84%

Abdominal TB	2	3.84%
Pleural Effusion	12	23.07%
Total	52	100

Table: 02: Descriptive Statistics of Urinary Neopterin among different groups:

Urinary Neopterin	N	Minimum	Maximum	Mean	Std. Deviation
Baseline	52	2.11	8.30	5.6700	1.82609
At 3 months	52	1.70	5.33	3.3350	.95985
At the end	52	1.80	3.80	2.6142	.56281
LTBI	24	1.81	3.07	2.2317	.42278
Controls	24	.0001	2.130	1.23284	.926725

Table: 03: Comparison of Urinary Neopterin at different time intervals:

Urinary Neopterin	N	Minimum	Maximum	Mean	Std.	95% CI_LL	95% CI_UL	p-value
Baseline	52	2.11	8.30	5.6700	1.82609	4.9324	6.4076	0.0023
At 3 months	52	1.70	5.33	3.3350	.95985	2.9473	3.7227	
At the end	52	1.80	3.80	2.6142	.56281	2.3429	2.8855	
Total	156	1.70	8.30	3.9972	1.82285	3.5657	4.4286	

Table: 04: Comparison of Urinary Neopterin among different groups using Post Hoc Analysis (Within groups):

Urinary Neopterin	Duration	Mean Difference	Std. Error	95% CI_LL	95% CI_UL	p-value
Baseline	**At 3 months**	2.33500*	.35610	1.4817	3.1883	.000
	At the end	3.05579*	.38752	2.1273	3.9843	.000
At 3 months	**Baseline'**	-2.33500*	.35610	-3.1883	-1.4817	.000
	At the end	.72079	.38752	-.2077	1.6493	.158
At the end	**Baseline**	-3.05579*	.38752	-3.9843	-2.1273	.000
	At 3 months	-.72079	.38752	-1.6493	.2077	.158

Table: 05: Comparison of Urinary Neopterin among different groups using Post Hoc Analysis (Within groups):

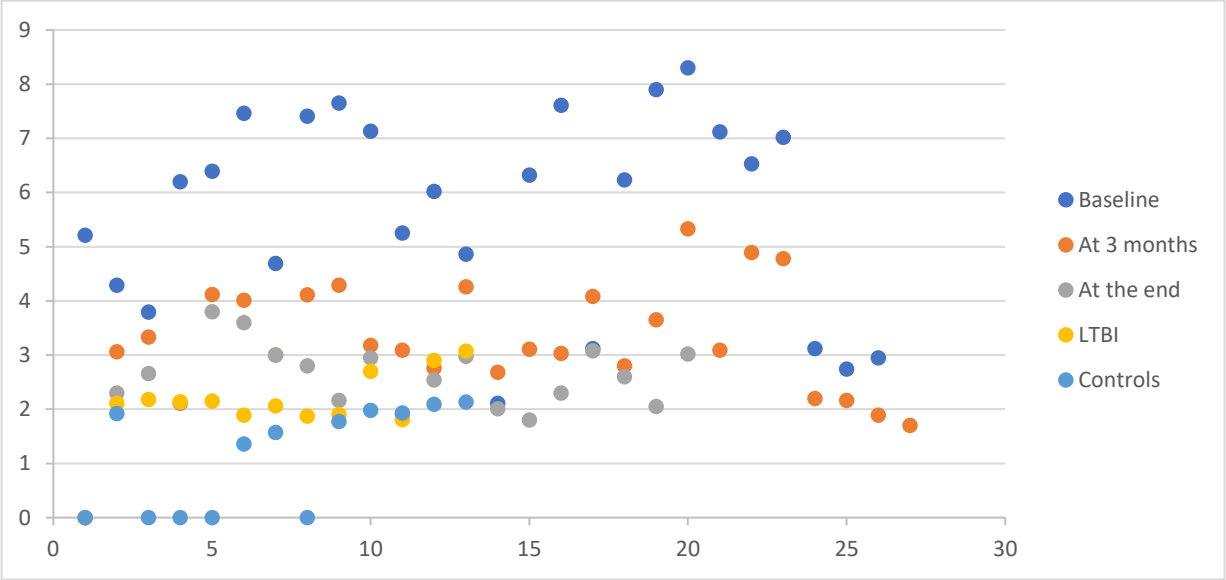
Urinary Neopterin in different groups	Statistics	Baseline	At 3-month follow-up	At the end of treatment	LTBI	Controls
Baseline	**Pearson Correlation**	1	.573**	.392	-.032	.620*
	p-value		.002	.097	.921	.032
At 3 month follow-up	**Pearson Correlation**	.573**	1	.517*	-.082	-.008
	p-value	.002		.023	.801	.981
At the end of treatment	**Pearson Correlation**	.392	.517*	1	.141	-.222
	p-value	.097	.023		.662	.488
LTBI	**Pearson Correlation**	-.032	-.082	.141	1	.402
	p-value	.921	.801	.662		.195

Controls	**Pearson Correlation**	.620*	-.008	-.222	.402	1
	p-value	.032	.981	.488	.195	

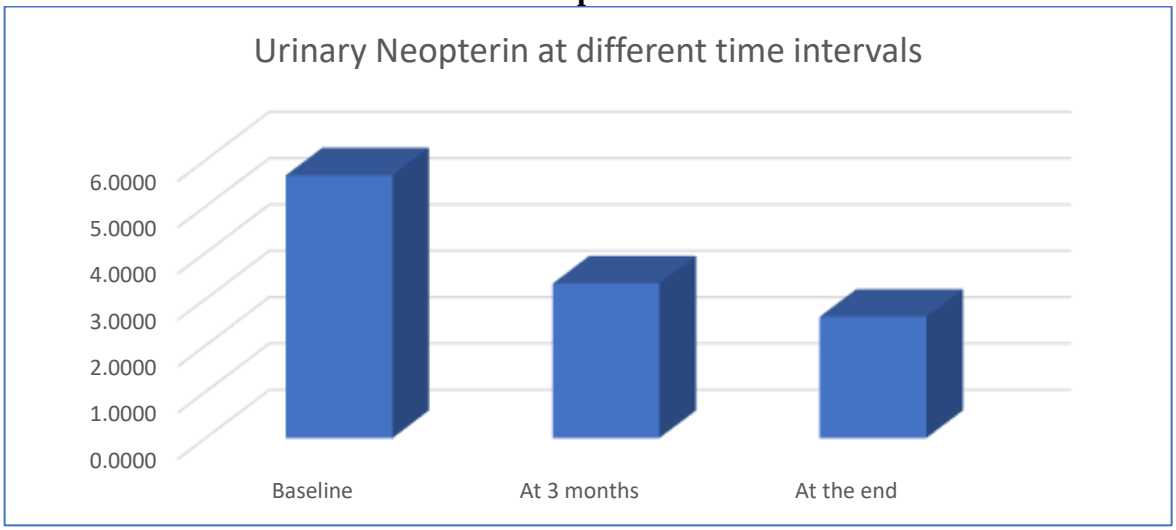
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

The above table illustrates the correlation for TB patients between the baseline, 3 months, at the end of treatment, LTBI and control subjects. It was found that baseline was positively correlated with at 3 months and controls along with statistically significant (p-value<0.05). While at 3 months was positively correlated and statistically significant (p-value<0.05) with baseline and at the end of treatment.

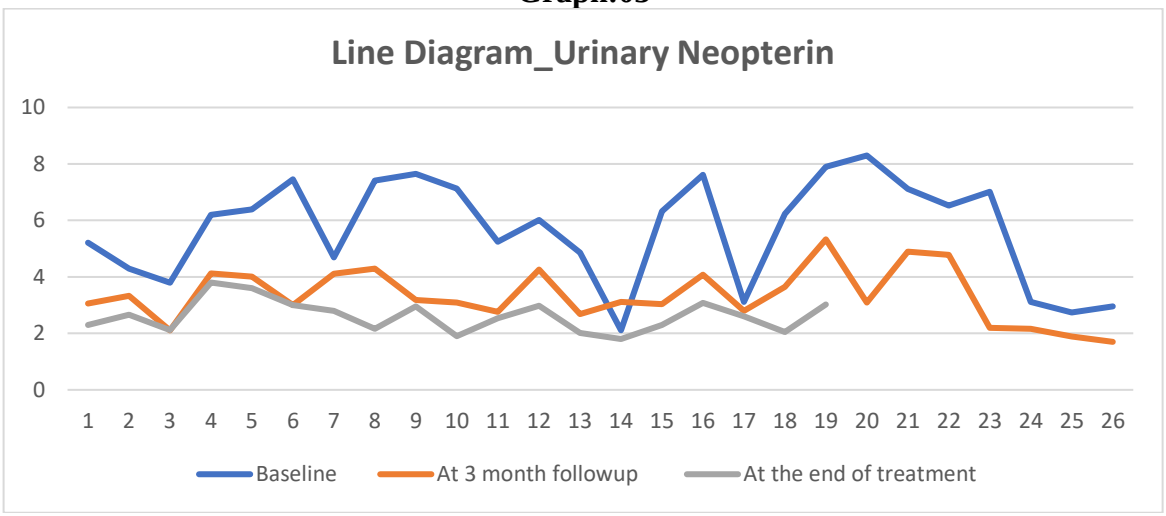
Graph:01



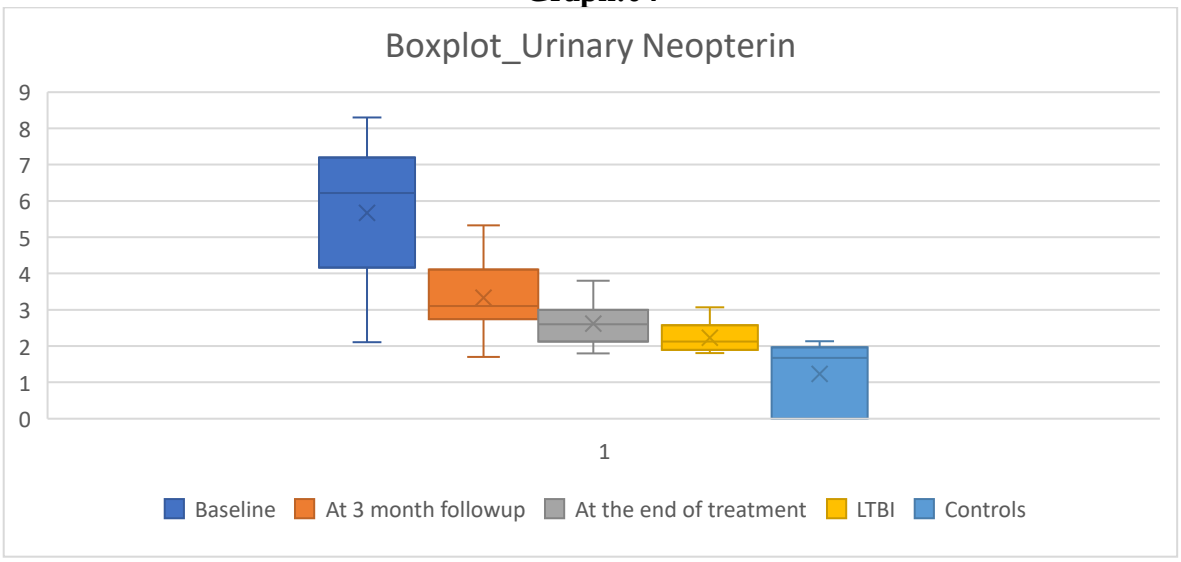
Graph:02



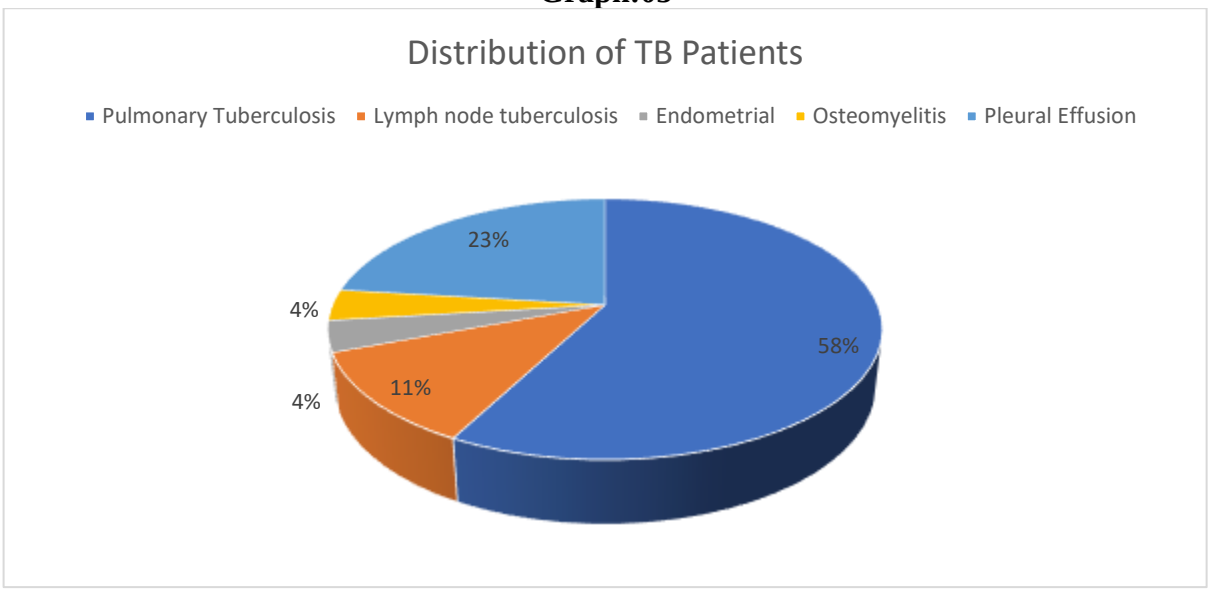
Graph:03



Graph:04



Graph:05



Discussion

Tuberculosis is a common infectious disease worldwide having both pulmonary and extra pulmonary manifestations. Majority of patients have pulmonary disease. Among extra pulmonary tuberculosis maximum cases are of pleural effusion, followed by lymph node tuberculosis. We have excelled in diagnosing pulmonary tuberculosis patients expectorating sputum and thus providing specimen for direct evidence or pathogenic biomarker evaluation for activity of disease, but biomarker for diagnosing non-productive pulmonary tuberculosis and extra pulmonary tuberculosis cases is lacking. If a combination of pathogen and host marker is made available resultant sensitivity and specificity of diagnosis of active tuberculosis can increase. Neopterin is a pteridine compound, a catabolic product of purine nucleotide guanosine triphosphate (GTP),is one of the biochemical markers known for assessment of cell mediated immune response. Neopterin is produced by body cells including macrophages and monocytes when activated mainly by interferon gamma. After production neopterin is secreted unaltered in urine. Neopterin can easily be analysed in biological fluids. It has been reported that neopterin can be used as sensitive biomarker in the diagnosis and prognosis of many diseases in which activated macrophages and T-cells have cross talk through interferon gamma. Tuberculosis is one of the representatives of such diseases, [5].

Lipoarabino mannan (LAM) estimation in urine has been successfully used to diagnose active tuberculosis but it's sensitivity and specificity is compromised in non HIV infected individuals [6,7]

Neopterin concentration in urine or serum seem to be of equal value for diagnostic application as long as renal function is normal. With improvement in nutritional status and rehydration, muscle mass increases and leads to increased catabolic activities producing more of creatinine which will adversely effect ratio of neopterin to creatinine [8].

Probably this is the first study in India to our knowledge investigating urinary level alone for diagnostic as well prognostic value of active tuberculosis.

In our study the mean age of the patient was 26.2 year, males are more as compared to female. Majority of patients are having pulmonary tuberculosis. Among extrapulmonary tuberculosis maximum cases are of pleural effusion (23.07%) followed by lymph node tuberculosis(11.53%). Our study compare the urinary neopterin value among active TB, latent TB and normal subjects. It was found that there was statistically significant difference in the base line urinary neopterin level among active cases (mean 5.67),LTBI (mean 2.23) and control (mean 1.23).Among active TB cases after the initiation of anti tubercular treatment (HRZE - HRE) follow up urinary neopterin also done at 3 months and at 6 months. It was found that comparing urinary neopterin from base line to 3 month and at 6 month it was statistically significant (p value<0.05). further more on comparison of urinary neopterine at 3 month and 6 month it was statistically insignificant (p value >0.05) using ANOVA post hoc analysis. The study on urinary neopterin to diagnose tuberculosis are very limited and mostly done on comparison of urinary neopterin creatinine ratio rather than urinary neopterin alone.

Michael Eisenhut et al done a comparison study on urinary neopterin creatinine ratio among active TB ,LTBI and control and found significant difference in neopterin creatinine ratio between active TB LTBI and control but no significant difference between LTBI and control [6]. Another study done by Flonza isa et al on urinary urinary diacetylspermine, uridopropionic acid, salisilic acid and neopterin and found that there is significant difference in the level of these metabolite between tubercular and non tubercular respiratory diseases [7].

Conclusion

Urinary neopterin is significantly higher in patient with active TB compared to person with latent infection which in turn are higher than noninfected person. Urinary neopterin level decreases significantly during the treatment . urinary neopterin can be considered as a surrogate marker to diagnose active TB and treatment outcome.

References

1. A. Harari, V. Rozot, F. Bellutti Enders et al., "Dominant TNFα +Mycobacterium tuberculosis-specific CD4+ T cell responses discriminate between latent infection and active disease," NatureMedicine,vol.17,no.3,pp.372–376,2011.
2. U. Sester, M. Fousse, J. Dirks et al., "Whole-blood flowcytometricanalysisofantigen-specificCD4T-cellcytokineprofiles distinguishes active tuberculosis from non-active states," PLoSONE,vol.6,no.3,ArticleIDe17813,2011.
3. M.Eisenhut,"Neopterin in diagnosis and monitoring of infectious diseases," Journal of Biomarkers, vol. 2013, Article ID 196432,10pages,2013.
4. J. Westermann, F. Thiemann, L. Gerstner et al., "Evaluation of a new simple and rapid enzyme-linked immunosorbent assay kit for neopterin determination," Clinical Chemistry and LaboratoryMedicine,vol.38,no.4,pp.345–353,2000.
5. Flavall E. A., Crone E. M., Moore G. A. & Gieseg S. P. (2008) Dissociation of neopterin and 7, 8-dihydroneopterin from plasma components before HPLC analysis, Journal of Chromatography B. 863, 167– 171. https://doi.org/10.1016/j.jchromb.2007.12.019 PMID: 18234568
6. S. D. Lawn, A. D. Kerkhoff, M. Vogt, R. Wood, Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: A descriptive study. Lancet Infect. Dis. 12, 201–209 (2012).
7. Y. Hanifa, L. Telisinghe, K. L. Fielding, J. L. Malden, G. J. Churchyard, A. D. Grant, S. Charalambous, The diagnostic accuracy of urine lipoarabinomannan test for tuberculosis screening in a South African correctional facility. PLOS ONE 10, e0127956 (2015)
8. Fuchs D, Jaeger H, Popescu M, Reibnegger G, Werner ER, Kaboth W, et al. Comparison of serum and urine neopterin concentrations in patients with HIV-1 infection. Clin Chim Acta Int J Clin Chem. 1990;187(2):125–130
9. I.Yuksekol,M.Ozkan,O.Akguletal.,"Urinary neopterin measurement as a non-invasive diagnostic method in pulmonary tuberculosis," International Journal of Tuberculosis and Lung Disease,vol.7,no.8,pp.771–776,2003.
10. Michael Eisenhut, DougalS.Hargreaves,2et al Journal of Biomarkers Volume 2016, Article ID 5643853, 6 pages http://dx.doi.org/10.1155/2016/5643853
11. Flonza Isa, Sean Collins et al F.Isa etal. / EBioMedicine 31 (2018) 157–165