A Prospective Study of the Talaromyces marneffei Mannoprotein Mp1p ELISA for Early Detection of talaromycosis in Patients with Advanced HIV Disease in Vietnam

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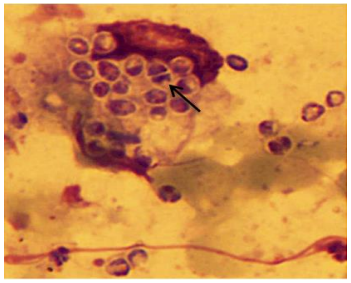
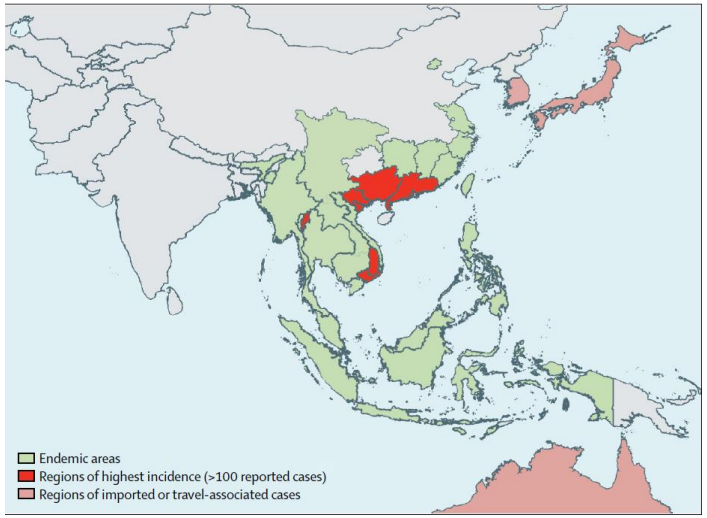
Disclosure

No conflict of interests

Burden of talaromycosis (penicilliosis)



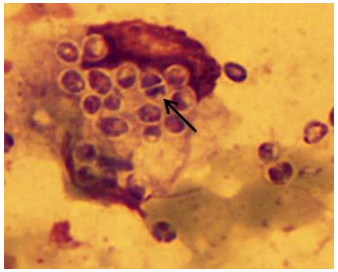


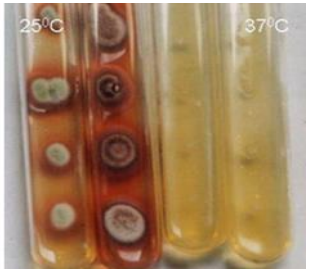
- A systematic fungal infection endemic in Southeast Asia
- A leading cause of HIV-associated death in Vietnam and southern China (1)
- Late diagnosis is the most challenging clinical problem and increases mortality from 24% to 50% (2)

(1) Fungal infections in HIV/AIDS, Lancet infection, 2017

(2) Hu, 2013, Mycopathologia

Current diagnostics for talaromycosis

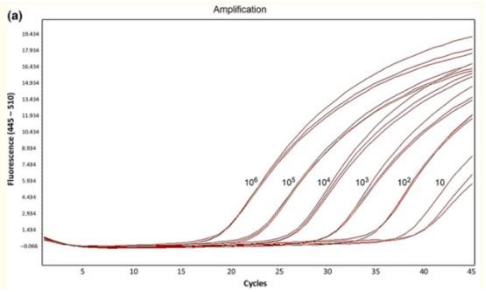




The Current Standard

Histopathology → invasive

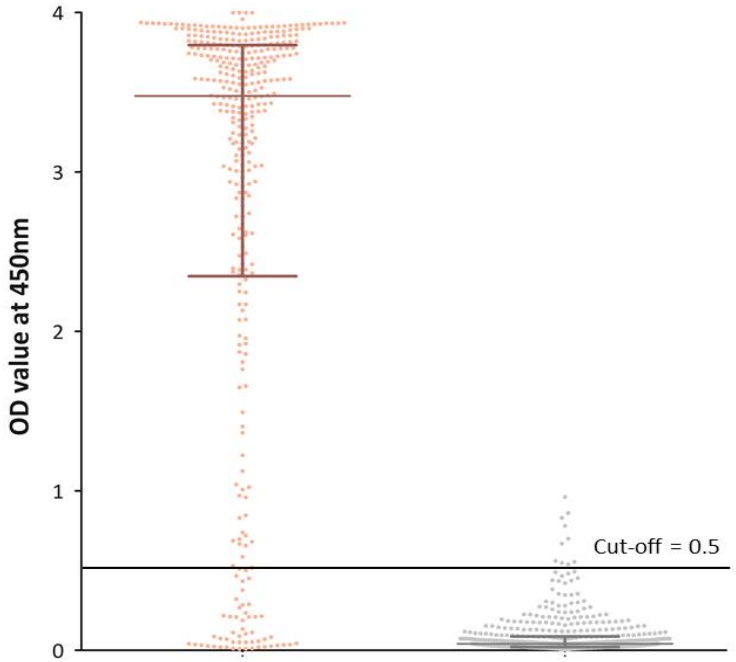
Cultures → incubation time up to 14 days; only 70% blood culture positive



Molecular: real time PCR → sensitivity is 70%; requires sophisticated machines and skills

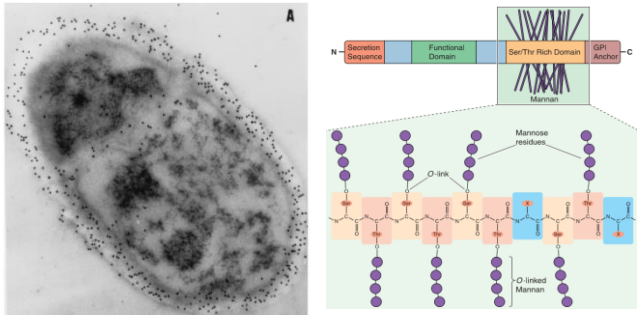
Serology: ELISA based antigen detection → simple; high sensitivity and specificity

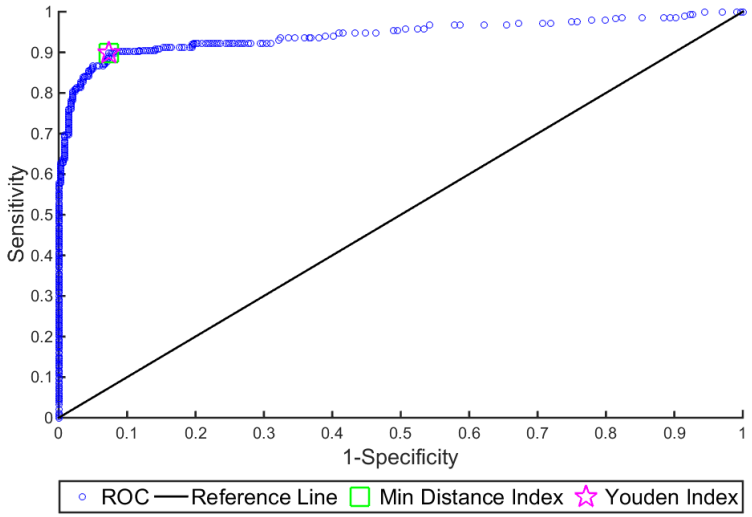
Mp1p ELISA



Culture-confirmed talaromycosis (N=372)

Non talaromycosis control (N=517)





Sensitivity: 86.4%

Specificity: 98%

Diagnostic AUC: 96%

Thu Nguyen, Poster at CROI 2017

Prevalence of Tm antigenemia in China and Vietnam

TABLE 1. Positive rates of Mp1p antigen in HIV-infected patients from Guangzhou during 2004–2011

Year	No. of samples	Mp1p antigen positive % (No. of samples)
2004	271	5.17 (14)
2005	527	6.45 (34)
2006	884	8.37 (74)
2007	894	9.62 (86)
2008	1221	9.42 (115)
2009	1284	10.28 (132)
2010	1256	12.58 (158)
2011	1794	8.25 (148)
Total	**8131**	**9.36 (761)**

TABLE 2. Prevalence of Mp1p antigenaemia in HIV-infected patient sera with different levels of CD4 count

CD4 count (cell/μL)	Mp1p antigen positive group (% no. of samples)	Mp1p antigen negative group (% no. of samples)	Total (no. of samples)
≥ 500	1.65 (6)	98.35 (357)	363
200 ~ 500	1.69 (17)	98.31 (991)	1008
100 ~ 200	4.53 (14)	95.47 (295)	309
50 ~ 100	10.36 (23)	89.63 (199)	222
< 50	28.06 (220)	71.94 (564)	784
Total	10.42 (280)	89.58 (2406)	2686

A

Provinces	N	Mp1p (+)
Northern sites	464	33 (6.6%)
Thai Nguyen	20	6 (23.1%)
Son La	28	3 (9.7%)
Nghe An	47	4 (7.8%)
Ha Noi	226	16 (6.6%)
Quang Ninh	30	2 (6.2%)
Vinh Phuc	36	2 (5.3%)
Thanh Hoa	77	0 (0%)
Southern sites	573	12 (2.1%)
An Giang	81	4 (4.7%)
HCM City	392	6 (1.5%)
Binh Duong	100	2 (2.0%)
Sum	**1082**	**45 (4.2%)**

Thu Nguyen, Poster at CROI 2019

Research Objectives

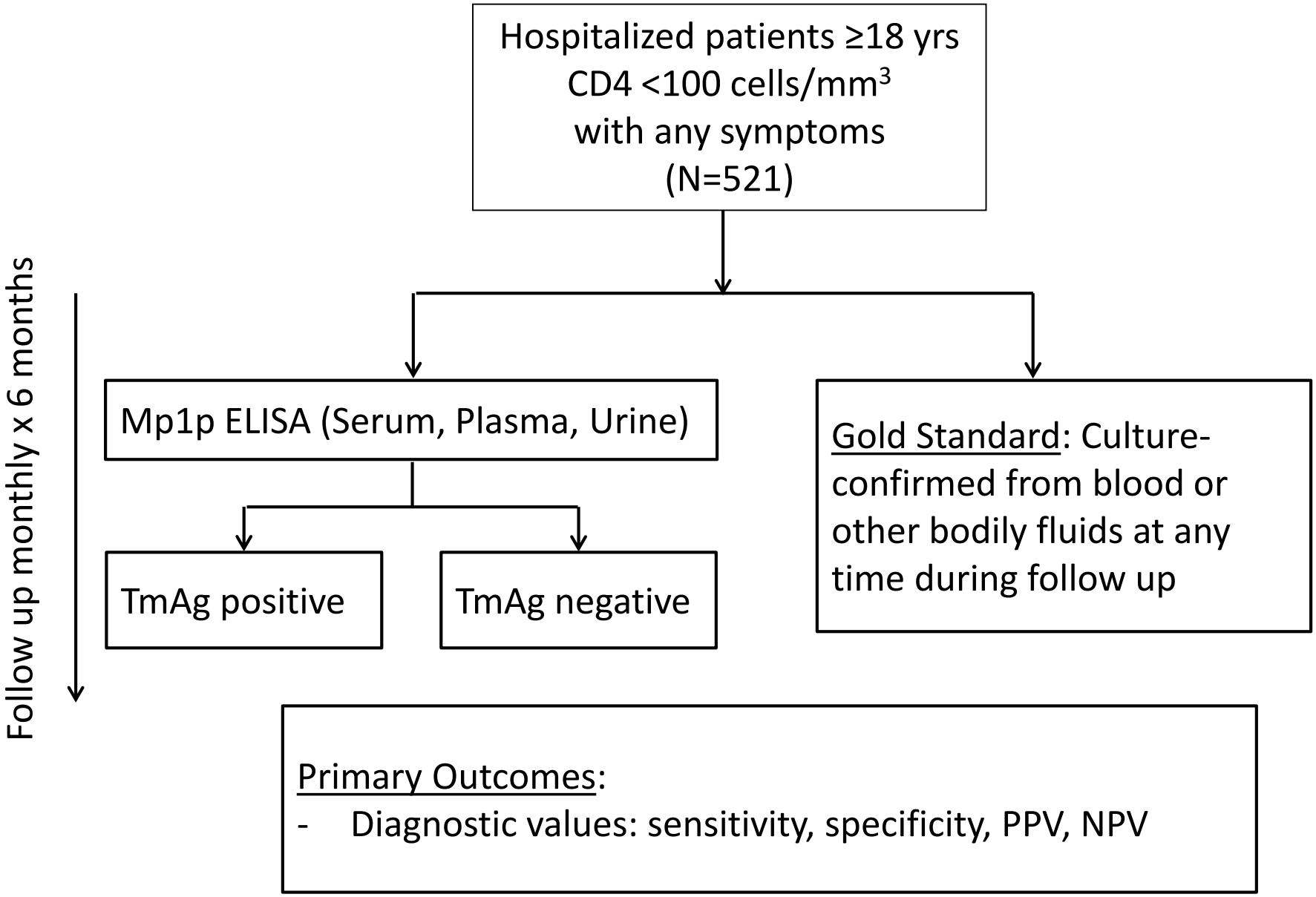
- To estimate the prevalence of Tm antigenemia in symptomatic hospitalized AIDS patients

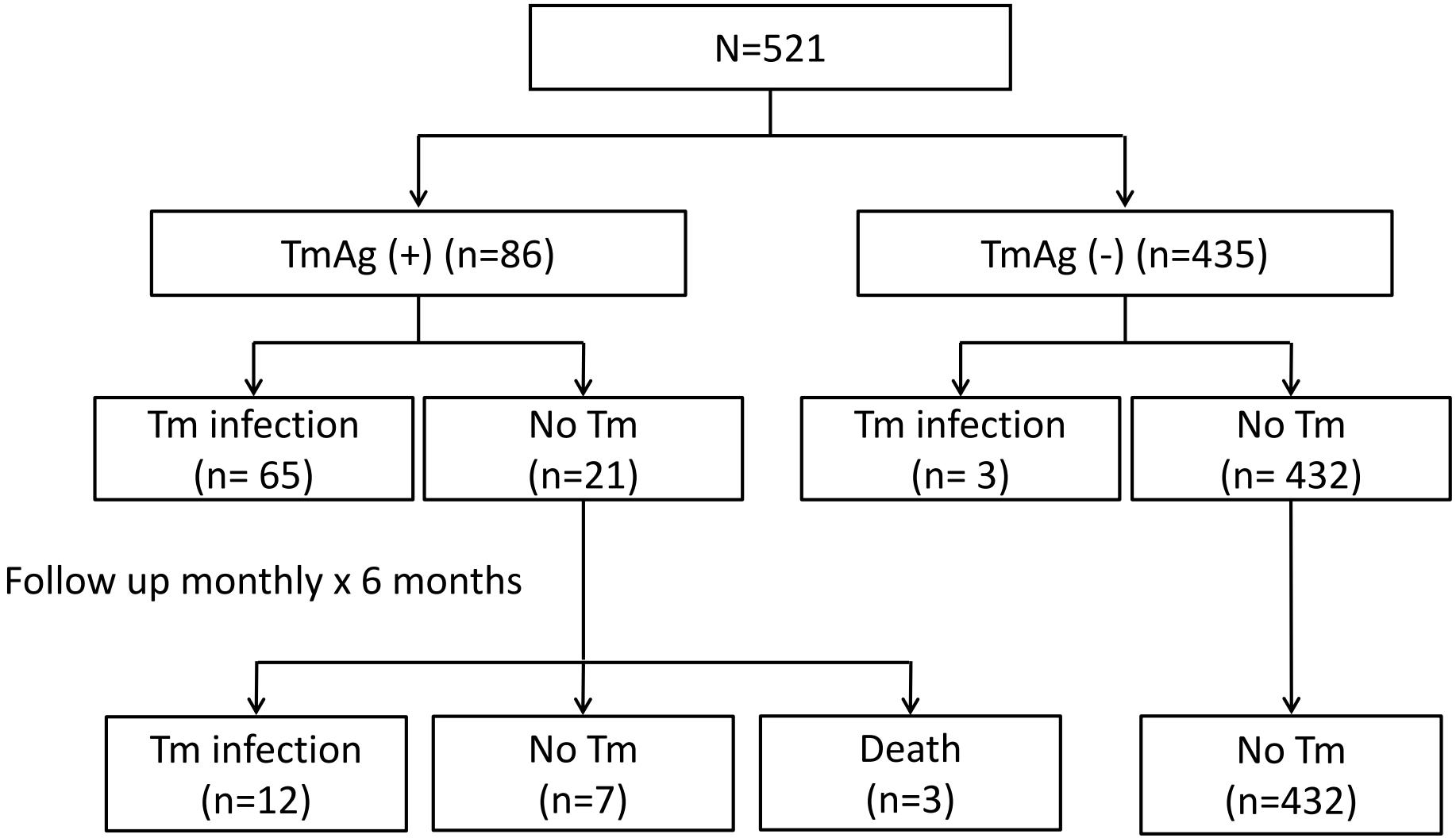
Hypothesis: prevalence is at least 10% in symptomatic AIDS patients

- To investigate the diagnostic values of Tm antigenemia to standard culture method

Hypothesis: Tm testing is more sensitive than blood culture and can detect infection earlier than culture

Methods


Hospitalized patients ≥18 yrs
CD4 <100 cells/mm3
with any symptoms
(N=521)
Follow up monthly x 6 months
Mp1p ELISA (Serum, Plasma, Urine)
TmAg positive
TmAg negative
Gold Standard: Culture-confirmed from blood or other bodily fluids at any time during follow up
Primary Outcomes:
- Diagnostic values: sensitivity, specificity, PPV, NPV



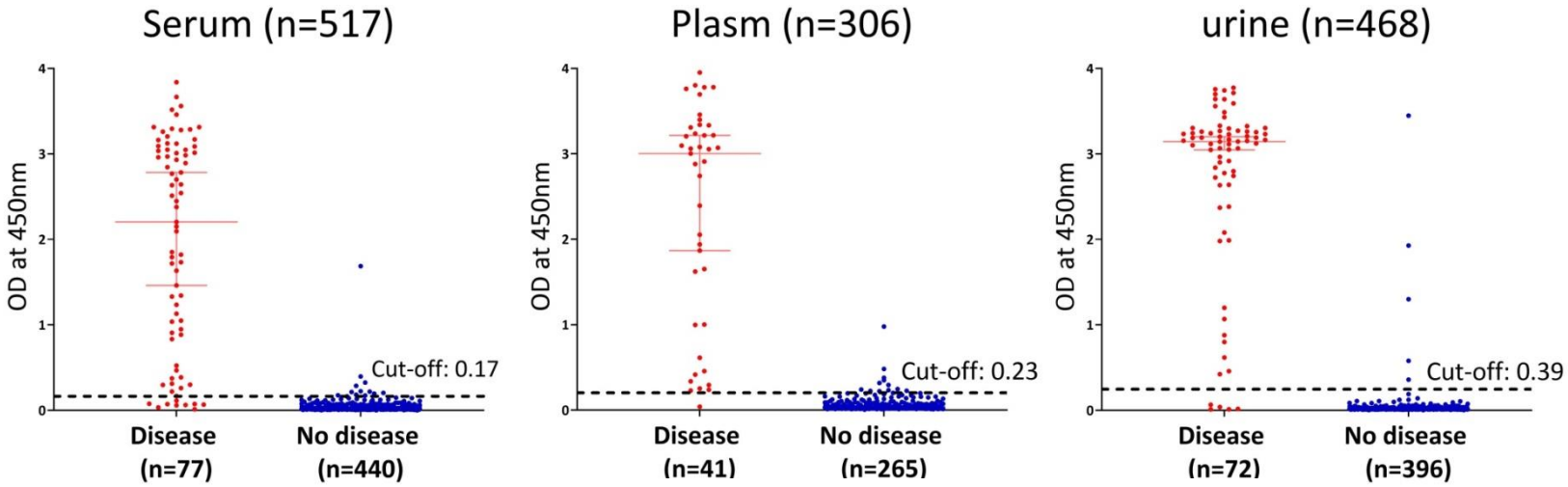
TmAg: *Talaromytces marneffei* antigenemia

Baseline characteristics

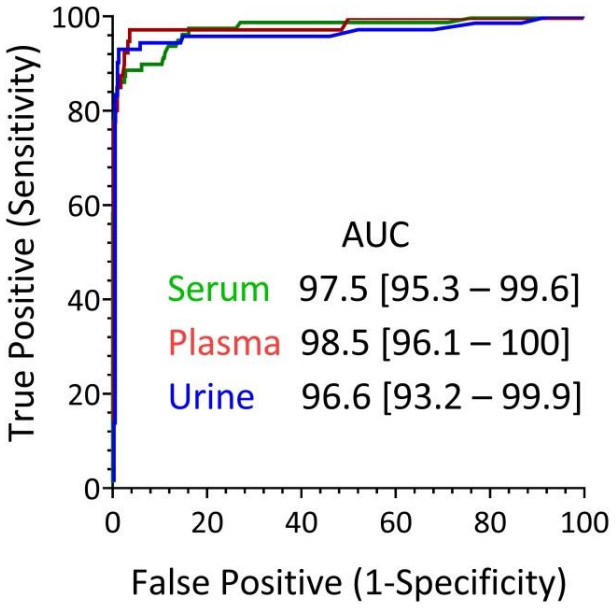
Characteristics	Total (n=504)	Tm (n=80)	No Tm (n=441)	p value
Age (years) n=499 (median [IQR])	34.0 [29.8 - 40.0]	32.5 [27.5 - 38.0]	35.0 n=419 [30.0 - 40.0]	0.059
CD4 count (cells/µl) n=498 (median [IQR])	17.0 [6.0 - 36.0]	12.0 [4.0 - 27.3]	17.0 n=418 [7.0 - 37.0]	0.033
Sex, male (%)	393 (78.6)	66 (82.5)	327 (77.9)	0.768
White Blood Count (x10^3/µL) n=499 (median [IQR])	5.7 [3.4 – 8.7]	4.1 [2.5 – 7.1]	6.0 n=419 [3.7 – 9.2]	<0.001
Haemoglobin (g/dL) n=499 (median [IQR])	10.0 [8.3 – 11.7]	8.8 [7.6 – 10.8]	10.2 n=419 [8.4 – 12.2]	<0.001
Platelet count (x10^3/µL) n=497 (median [IQR])	201 [122 – 301]	100 n=79 [49.5 – 165.5]	224.5 n=418 [143 – 312]	< 0.001
Creatinine (µmol/L) n=477 (median [IQR])	71 [55 – 86]	76 n=71 [58 – 91.5]	70 n=406 [54 – 85]	0.182
AST (Units/L) n=470 (median [IQR])	55 [32 – 99]	126 n=77 [83 – 246]	48 n=393 [29 – 78]	< 0.001
ALT (Units/L) n=470 (median [IQR])	35.5 [23 – 66.8]	55 n=77 [30 – 91]	33 n=393 [22 – 61]	0.001

Abbreviations: IQR, interquartile range
Categorical variables: n (%). Continuous variables (data not normally distributed): median [IQR]

OD value distribution in sera, plasma, and urine samples of Tm and non Tm patients



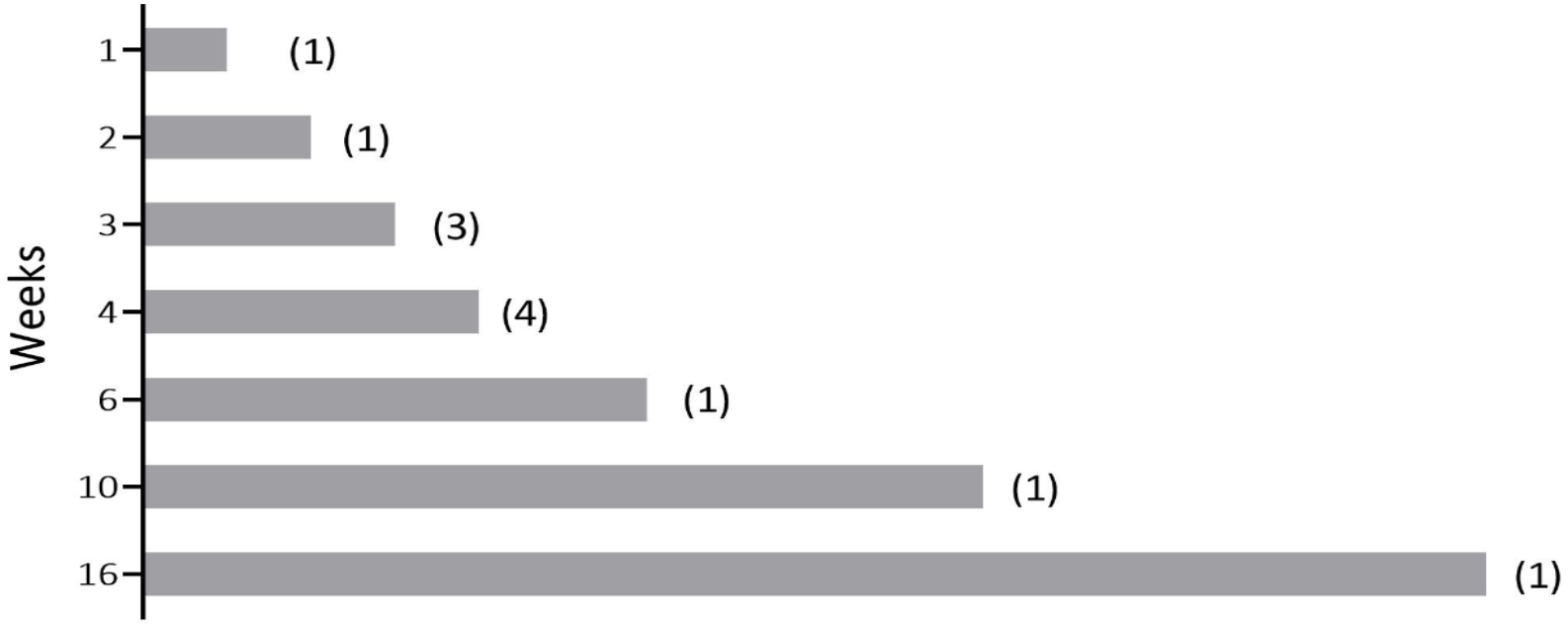
The ROC curves for sera, plasma, and urine



Sensitivities between TmAg vs. positive cultures at enrollment

	Culture-confirmed		Sum	*p* value
TmAg	Pos	Neg		
Pos	65	13	78 (97.5%)	0.005 (McNemar test)
Neg	2	0	2	
Sum	67 (83.8%)	13	80	

Time to development of Tm infection in 12 TmAg (+) culture (-) patients



Diagnostic performance in sera, plasma, and urine samples of Tm and non Tm patients

N=521	Serum	Plasm	Urine	*p* value[a]
AUC	97.5% [95.3 – 99.6] AUC_{Serum}-AUC_{Plasma} p=0.54	98.5% [96.1 – 100] AUC_{Plasma} vs AUC_{Urine} p=0.36	96.6% [93.2 – 99.9] AUC_{Urine} vs AUC_{Serum} p=0.66	(b)
Sensitivity	87.1% [77.7 – 92.8]	97.6% [87.4 – 99.9]	93.1% [84.8 – 97.0]	0.33
Specificity	97.3% [95.3 – 98.4]	96.6% [93.7 – 98.2]	99.0% [97.4 – 99.6]	0.071
PPV	84.8% [75.3 – 91.1]	81.6% [68.6 – 90.0]	94.4% [86.4 – 97.8]	
NPV	97.7% [95.9 – 98.8]	99.6% [97.8 – 100]	98.7% [97.1 – 99.5]	

[a] Fisher's exact test

[b] Delong's test

Conclusions

- Prevalence of TmAg in symptomatic patients with CD4 <100 was 16.5%
- Tm antigen test is superior to conventional cultures (96% vs. 83%) for diagnosing Tm infection
- TmAg can be detected up to 16 weeks before any cultures becomes positive
- Sensitivities were similar in sera, plasma, and urine
- 70% of TmAg (+) culture (-) patients developed infection or died during the follow up
- A commercial Tm antigen test has just been approved for clinical diagnostic in China

Acknowledgements