



**University of Modena
& Reggio Emilia**

*Section of Respiratory Diseases,
Policlinico Hospital, Modena*

***Using Interferon- γ Release Assays
Day by Day in a Hospital Setting***

Giovanni Ferrara

***First Global Symposium on IFN- γ assays
Vancouver, February 22^o 2007***



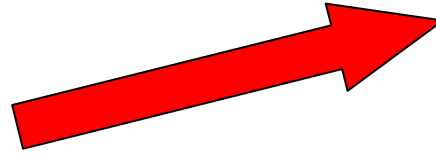
Diagnosing TB infection with IGRAs...

- **INTRODUCTION**
- **FIRST EXPERIENCE**
- **HEAD to HEAD STUDY**
- **CONCLUSIONs...**



SPECIFICITY

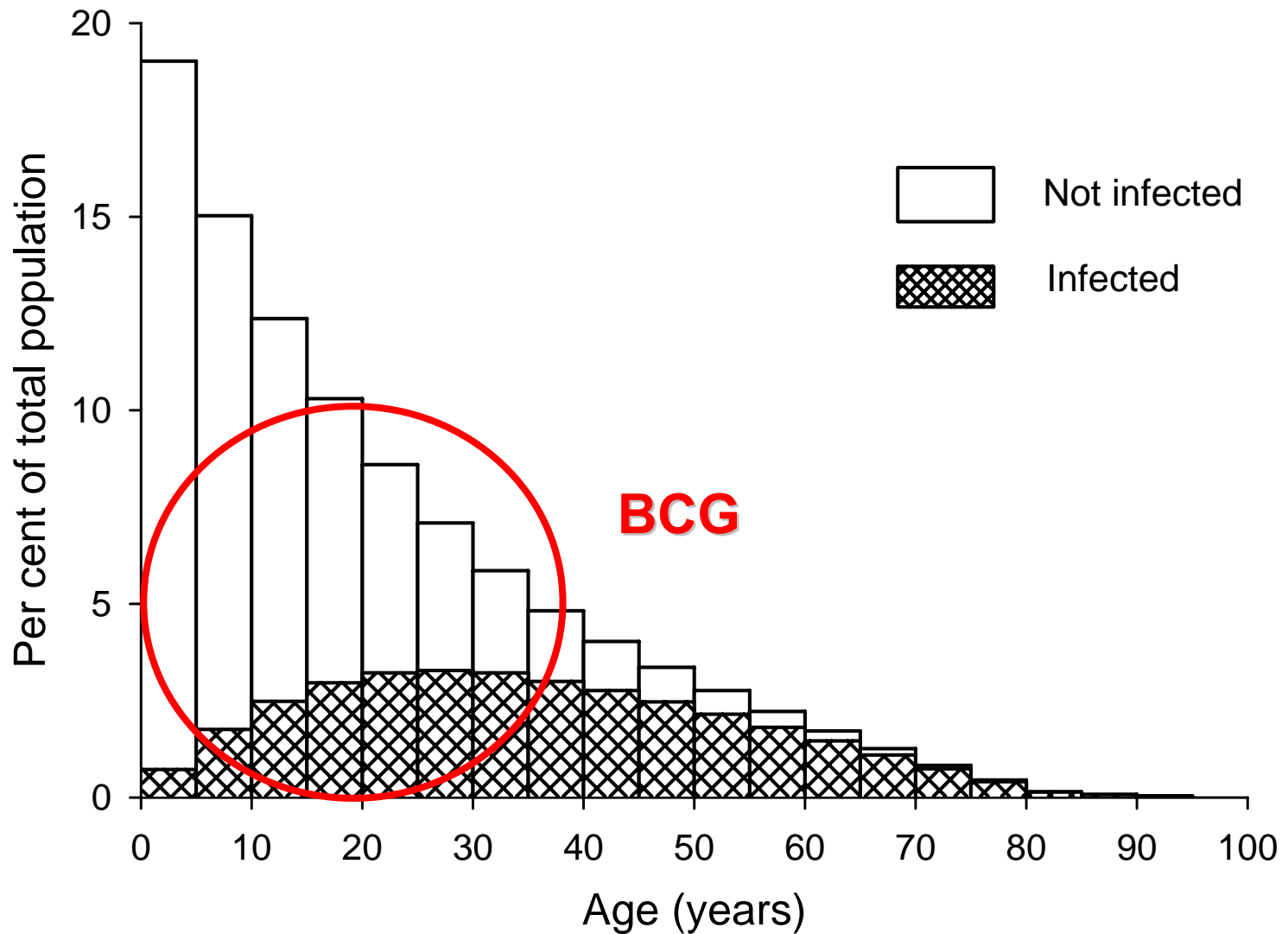
**False positive
(BCG, NTM)**



**Drawbacks of
the TST**



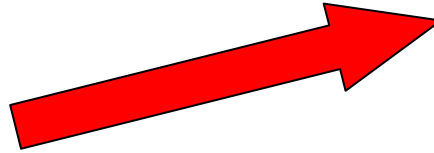
Age-specific Prevalence of Tuberculous Infection in Tropical and Southern Africa



Data courtesy: ten Dam HG, World Health Organization, 1990

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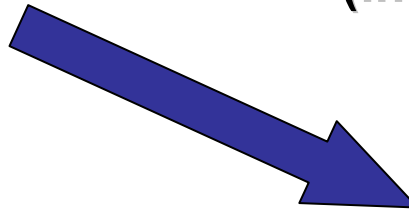


SENSITIVITY

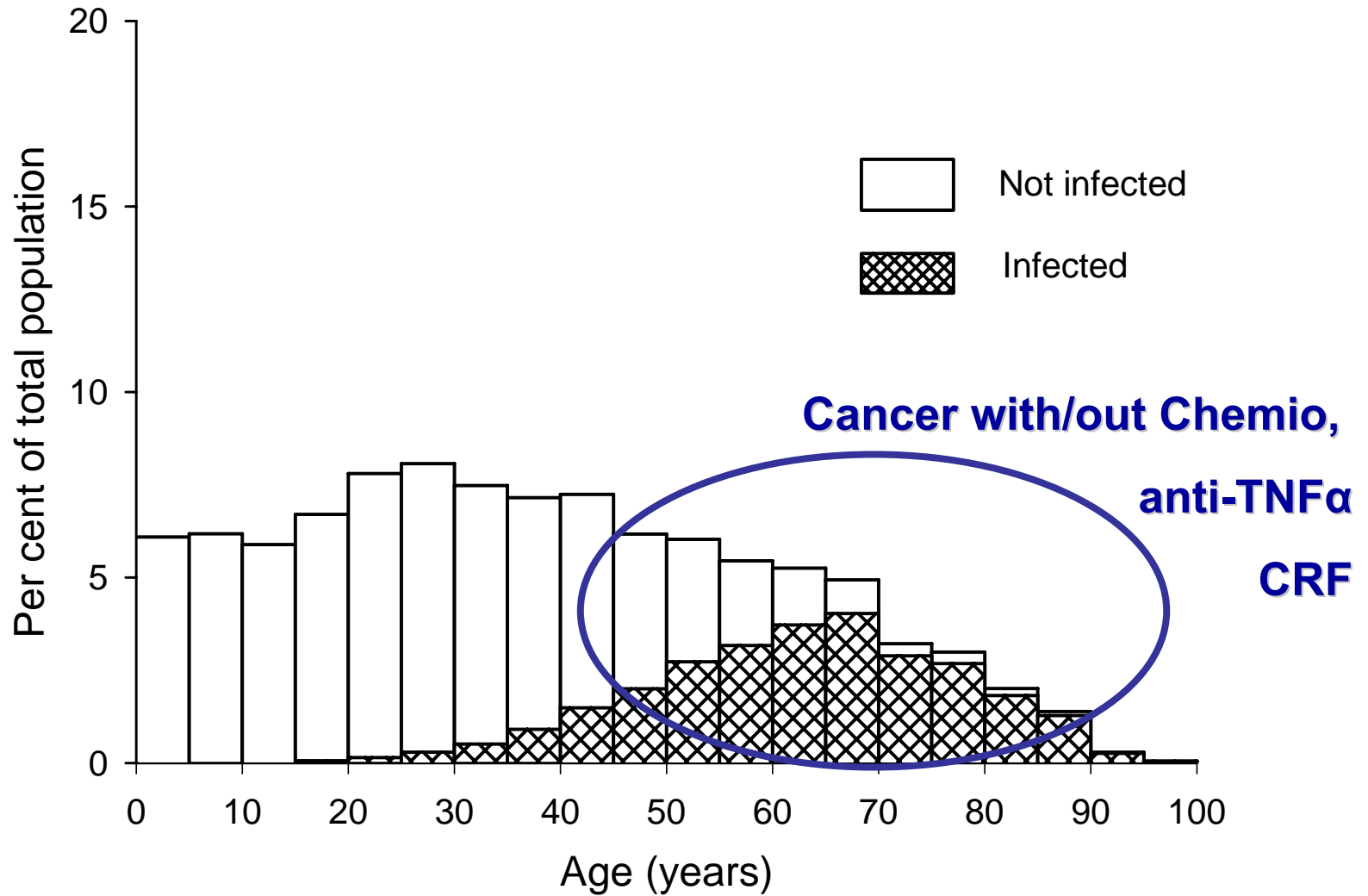
**False negative
(impaired immune response):**

- HIV
- Immunosuppressive therapies
- Age
- CRF, Cancer

**Drawbacks of
the TST**



Age-specific Prevalence of Tuberculous Infection in Western Europe



Data courtesy: ten Dam HG, World Health Organization, 1990

Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection

CRITERIA FOR TUBERCULIN POSITIVITY, BY RISK GROUP

Reaction \geq 5 mm of Induration	Reaction \geq 10 mm of Induration	Reaction \geq 15 mm of Induration
Human immunodeficiency virus (HIV)-positive persons	Recent immigrants (i.e., within the last 5 yr) from high prevalence countries	Persons with no risk factors for TB
Recent contacts of tuberculosis (TB) case patients	Injection drug users	
Fibrotic changes on chest radiograph consistent with prior TB	Residents and employees ¹ of the following high-risk congregate settings: prisons and jails, nursing homes and other long-term facilities for the elderly, hospitals and other health care facilities, residential facilities for patients with acquired immunodeficiency syndrome (AIDS), and homeless shelters	
Patients with organ transplants and other immunosuppressed patients (receiving the equivalent of \geq 15 mg/d of prednisone for 1 mo or more)*	Mycobacteriology laboratory personnel Persons with the following clinical conditions that place them at high risk: silicosis, diabetes mellitus, chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignancies (e.g., carcinoma of the head or neck and lung), weight loss of \geq 10% of ideal body weight, gastrectomy, and jejunioileal bypass Children younger than 4 yr of age or infants, children, and adolescents exposed to adults at high-risk	

* Risk of TB in patients treated with corticosteroids increases with higher dose and longer duration.

¹For persons who are otherwise at low risk and are tested at the start of employment, a reaction of \geq 15 mm induration is considered positive.

Source: Adapted from Centers for Disease Control and Prevention. Screening for tuberculosis and tuberculosis infection in high-risk populations: recommendations of the Advisory Council for the Elimination of Tuberculosis. M.M.W.R. 1995;44(No. RR-11):19-34.



THE PERFECT TEST FOR TB INFECTION SHOULD BE...

- **MORE SENSITIVE** THAN THE TST, ESPECIALLY AMONG **IMMUNO-COMPROMISED SUBJECTS**
- **MORE SPECIFIC**, ESPECIALLY AMONG **BCG VACCINATED INDIVIDUALS**
- FAST, EASY AND UNEXPENSIVE
- ABLE TO DISCERN **LTBI AND ACTIVE DISEASE, REMOTE AND RECENT INFECTION** AND TO MONITOR THE **TREATMENT OUTCOME**



	T-SPOT.TB	QuantiFERON-TB Gold	TST
Antigens:	ESAT-6 and CFP10	ESAT-6 and CFP10	PPD
Positive control:	Yes	Yes	No
Standard procedure:	Yes	Yes	No
Boosting effect:	No	No	Yes
Return visit:	No	No	Yes
Time for the result:	16-20 hours	16-24 hours	48-72 hours
Typology:	<i>In vitro</i>	<i>In vitro</i>	<i>In vivo</i>
Reading:	Instrumental	Instrumental	By the operator
Readout:	IFN-γ SFU	IFN-γ IU	Mm of induration
Platform:	ELISPOT	ELISA	/
Substrate:	PBMCs	Whole blood	/
Controlled Cell count:	Yes	No	/
Instruments:	ELISPOT counter	ELISA reader	/

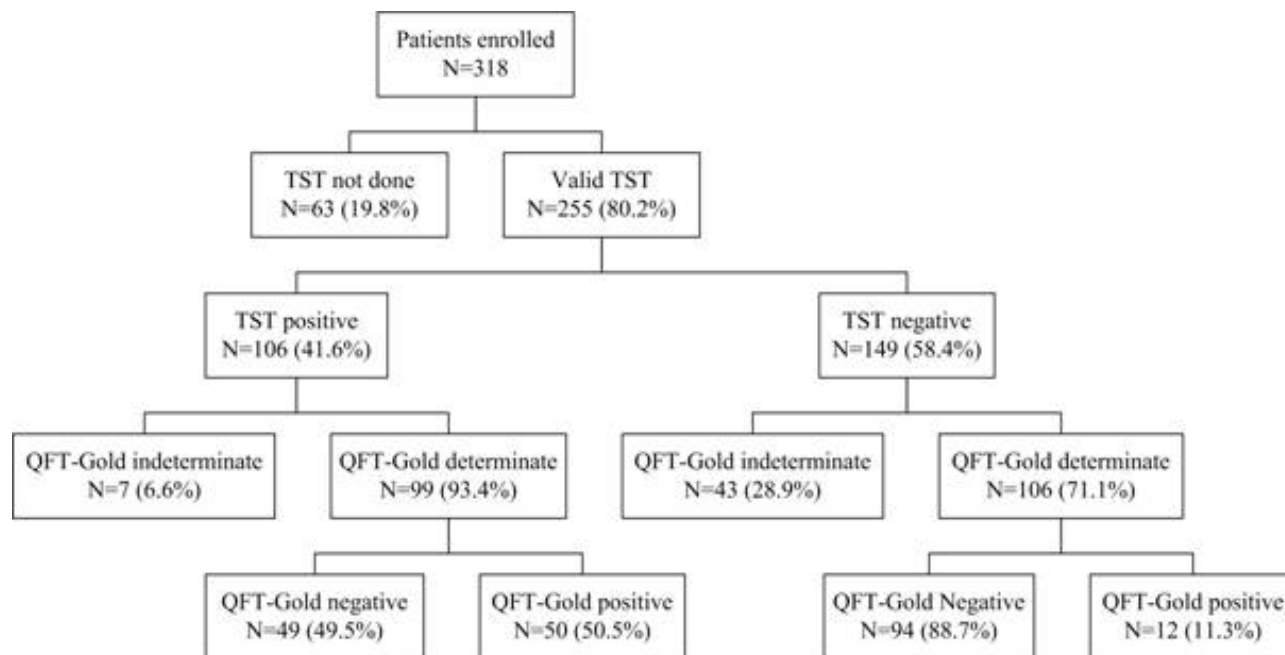


Diagnosing TB infection with IGRAs...

- INTRODUCTION
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- CONCLUSION...



Routine Hospital Use of a New Commercial Whole Blood Interferon- γ Assay for the Diagnosis of Tuberculosis Infection



Agreement with the TST

Overall: κ 0.40 (0.27-0.52)

BCG: κ 0.09 (0.05-0.23)

Routine Hospital Use of a New Commercial Whole Blood Interferon- γ Assay for the Diagnosis of Tuberculosis Infection

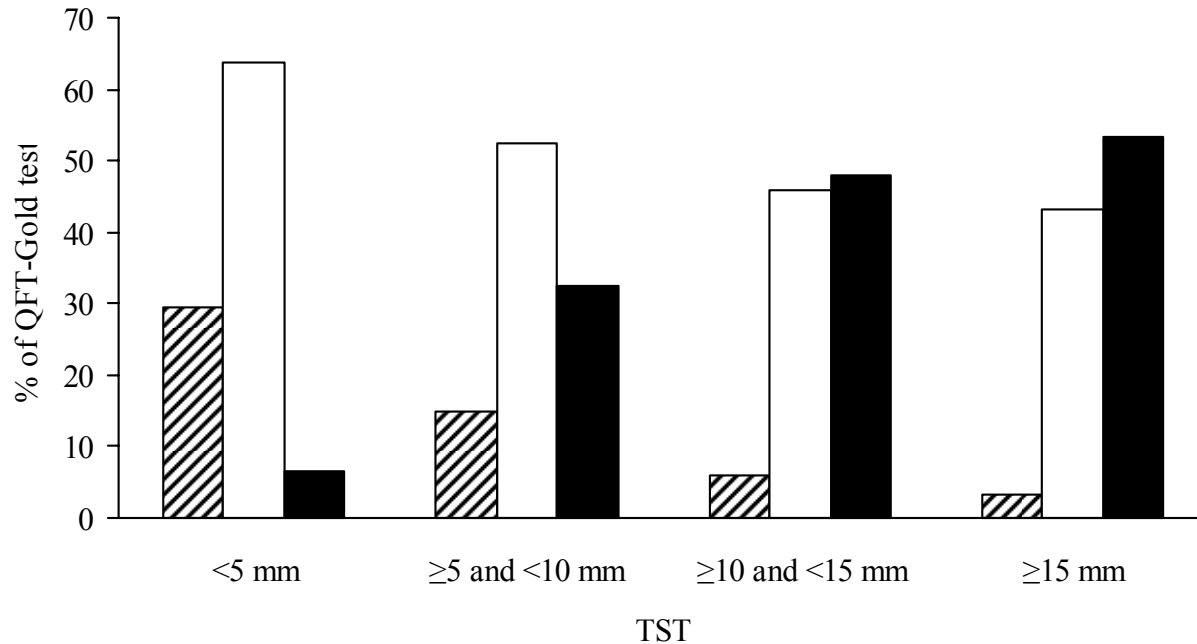
21.4% of tests with an indeterminate result

Variable	QFT-Gold		OR (95% CI)	p value
	Indeterminate* n = 68 (%)	Determinate n = 250 (%)		
Immunosuppressive therapy†	26 (38.2)	39 (15.6)	3.35 (1.84-6.08)	0.00007
Diagnosis of cancer‡	8 (11.8)	33 (13.2)	1.07 (0.49-2.30)	0.855
Age <3 or >80 years	11 (16.2)	26 (10.4)	1.46 (0.68-3.12)	0.324
HIV infection	2 (2.9)	5 (2.0)	2.53 (0.55-11.59)	0.230
Renal failure	4 (5.8)	6 (2.4)	2.26 (0.62-8.25)	0.214

† cancer chemotherapy, systemic steroids, anti-TNF alpha agents (n = 3)

‡Patients with a diagnosis of cancer who were not receiving anti-cancer treatments.

Routine Hospital Use of a New Commercial Whole Blood Interferon- γ Assay for the Diagnosis of Tuberculosis Infection



Indeterminate results associated with a negative TST



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HEAD TO HEAD
T-SPOT.TB (TS.TB)
VS
QuantiFERON TB Gold (QFT-G)



HEAD TO HEAD

T-SPOT.*TB* (TS.*TB*)

VS

QuantiFERON TB Gold (QFT-G)

Aims

- To compare the performance of the 2 tests (indeterminates and associated factors)
- To compare the 2 tests in active TB forms and groups at higher risk
- To compare the 2 blood tests with the TST (BCG vaccinated)



HEAD TO HEAD

T-SPOT.TB (TS.TB)

VS

QuantiFERON TB Gold (QFT-G)

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Methods

Prospective enrollment of all the patients tested at the Policlinico of Modena, Italy

Tests performed at the Microbiology Laboratory by 2 skilled technicians

Clinical data obtained from clinical charts and databases



RESULTS

STUDY POPULATION (October 2004- August 2005)

N=393 patients

Demographics	N (%)
Male	212 (53.9)
Age (mean - range)	43 (0 - 93)
BCG vaccinated	72 (18.3)
Cancer patients	69 (17.6)
Undergoing Chemotherapy	28 (7.1)
Undergoing Systemic Steroids	35 (8.9)
Undergoing anti-TNF-α	10 (2.5)
Age ≤ 5	25 (6.4)
Age ≥ 80	26 (6.6)

REASON TO TEST

Features	N (%)
Suspicion of active TB	166 (42)
Recent TB contact	119 (30)
Recent immigration	48 (12)
Screening before transplantation	9 (2)
Other reason	51 (13)
<i>Total</i>	<i>393 (100)</i>

Wards

Respiratory Diseases: 184 (46.8%) Paediatrics= 96 (24.4%)

Onco-Haematology: 53 (13.5%)



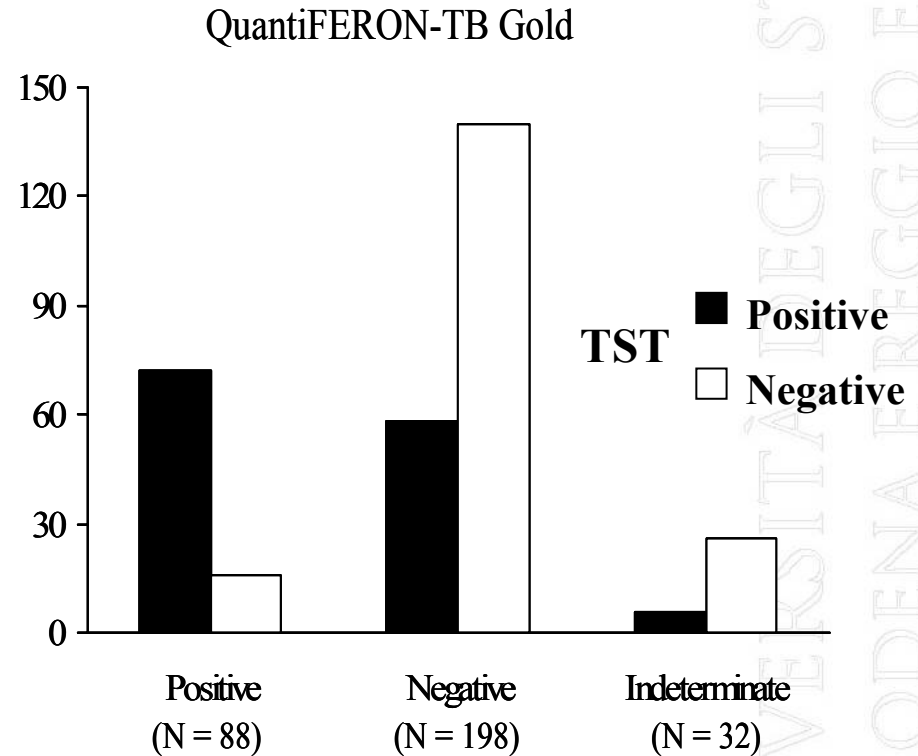
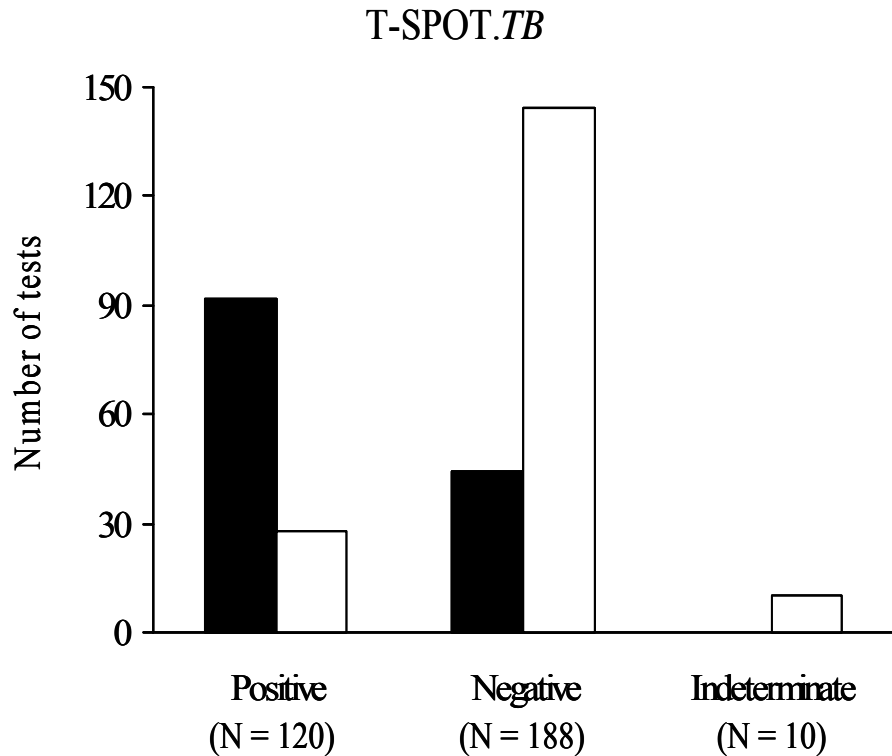
AGREEMENT BETWEEN TS.TB & QFT-G

		TS.TB			Total
		Positive	Negative	Indeterm.	
QFT-G	Positive	94	6	0	100
	Negative	40	191	9	240
	Indeterm.	10	30	3	43
Total		144	227	12	383

Total	K=0.699
Not-BCG	K=0.724
BCG	K=0.612



AGREEMENT WITH TST



Total (n = 308)	K=0.520
Not-BCG (n = 240)	K=0.628
BCG (n = 68)	K=0.133

*

Total (n = 286)	K=0.464
Not-BCG (n = 217)	K=0.615
BCG (n = 69)	K=0.122

*

*p<0.001

SAME PERFORMANCE?

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INDETERMINATE RESULTS

**ARE THEY ASSOCIATED WITH
IMMUNO-SUPPRESSION?**

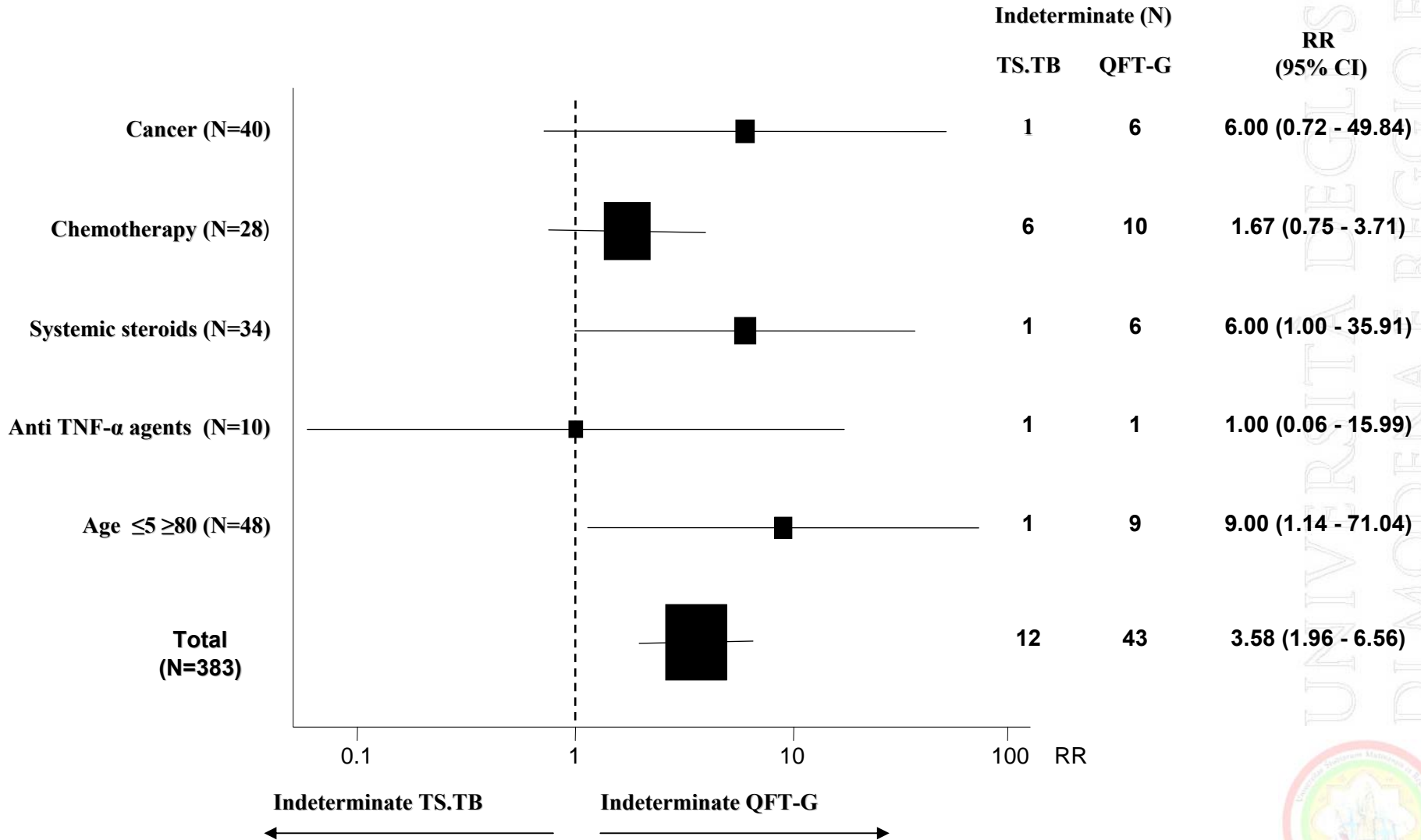


INDETERMINATE RESULTS

	QFT-G		TS.TB	
	OR	<i>p</i>	OR	<i>p</i>
Immuno-suppression	3.37	< 0.001	5.61	0.001
Chemotherapy	5.42	< 0.001	15.68	< 0.001
Age <5	4.21	0.003	0.57	0.593



INDETERMINATE RESULTS



POSITIVE RESULTS

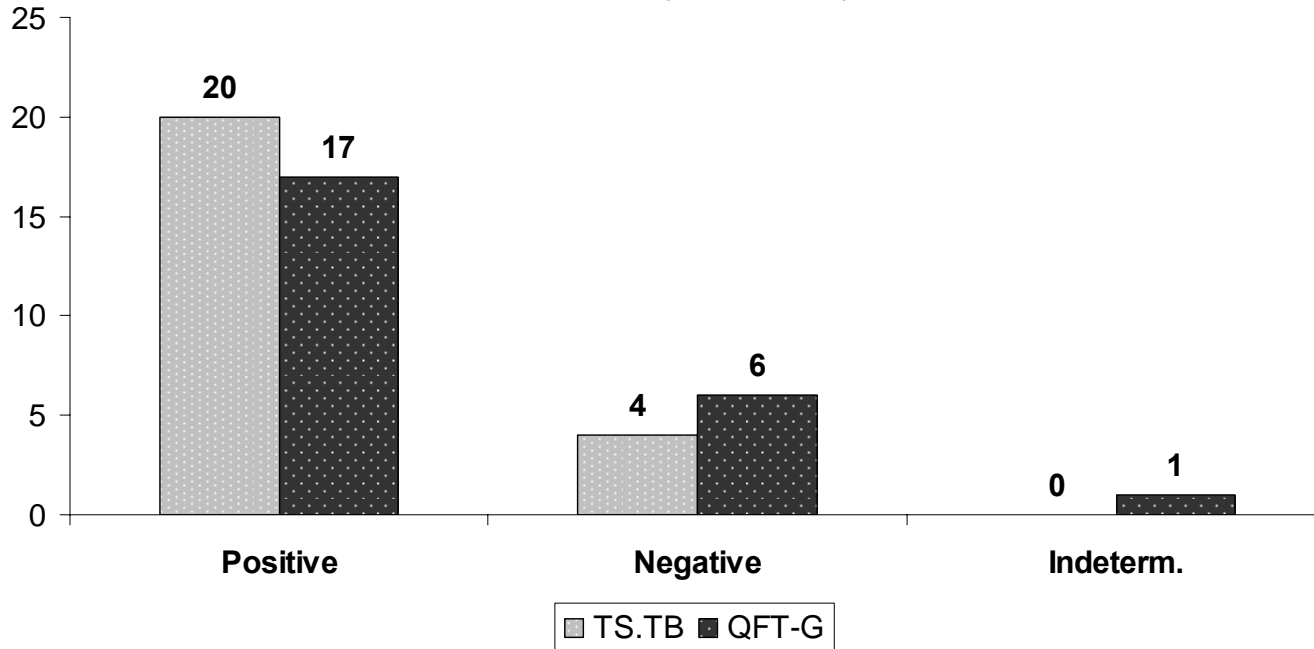
**WHAT ABOUT SURROGATE
“GOLD STANDARDS”
OF TB INFECTION?**



POSITIVE RESULTS

ACTIVE TB CASES

(N=24)



11 culture-confirmed cases

11 extrapulmonary forms, all TS.TB +, 8 QFT-G +

POSITIVE RESULTS

RECENT TB CONTACTS

(N=119)

		TS.TB	QFT-G
Positive results		39 (34%)	25 (22%)
		p<0.001	



POSITIVE RESULTS

RECENT TB CONTACTS

(N=119)

	TST	TS.TB	QFT-G
Positive results	62 (54%)	39 (34%)	25 (22%)
	p<0.001 vs TST		

POSITIVE RESULTS

RECENT TB CONTACTS

(N=119)

	TST	TS.TB	QFT-G
Positive results	62 (54%)	39 (34%)	25 (22%)
		p<0.001 vs TST	
Excluding BCG-vaccinated			
Positive results	36 (43%)	29 (35%)	21 (25%)
(N=87)		p=0.092	P=0.001

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CONCLUSIONs

**BOTH TESTS SHOWED A GOOD AGREEMENT
WITH THE TST AND HIGHER
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THE AGREEMENT BETWEEN THE BLOOD TESTS WAS HIGH, BUT THEY SHOWED SIGNIFICANT DIFFERENCES FOR:



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THE AGREEMENT BETWEEN THE BLOOD TESTS WAS HIGH, BUT THEY SHOWED SIGNIFICANT DIFFERENCES FOR:

- **INDETERMINATE RESULTS** (associated with different factors, as age)
- **POSITIVE RESULT** (discordant results with the TST)



A COMBINED APPROACH...



**...COULD BE THE BEST
CONSIDERING THE AVAILABLE EVIDENCE!**



Acknowledgments

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Paediatrics

Barbara Bergamini

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