

Invaziv Fungal Enfeksiyonlarda Tanı

Prof Dr Zekaver Odabaşı

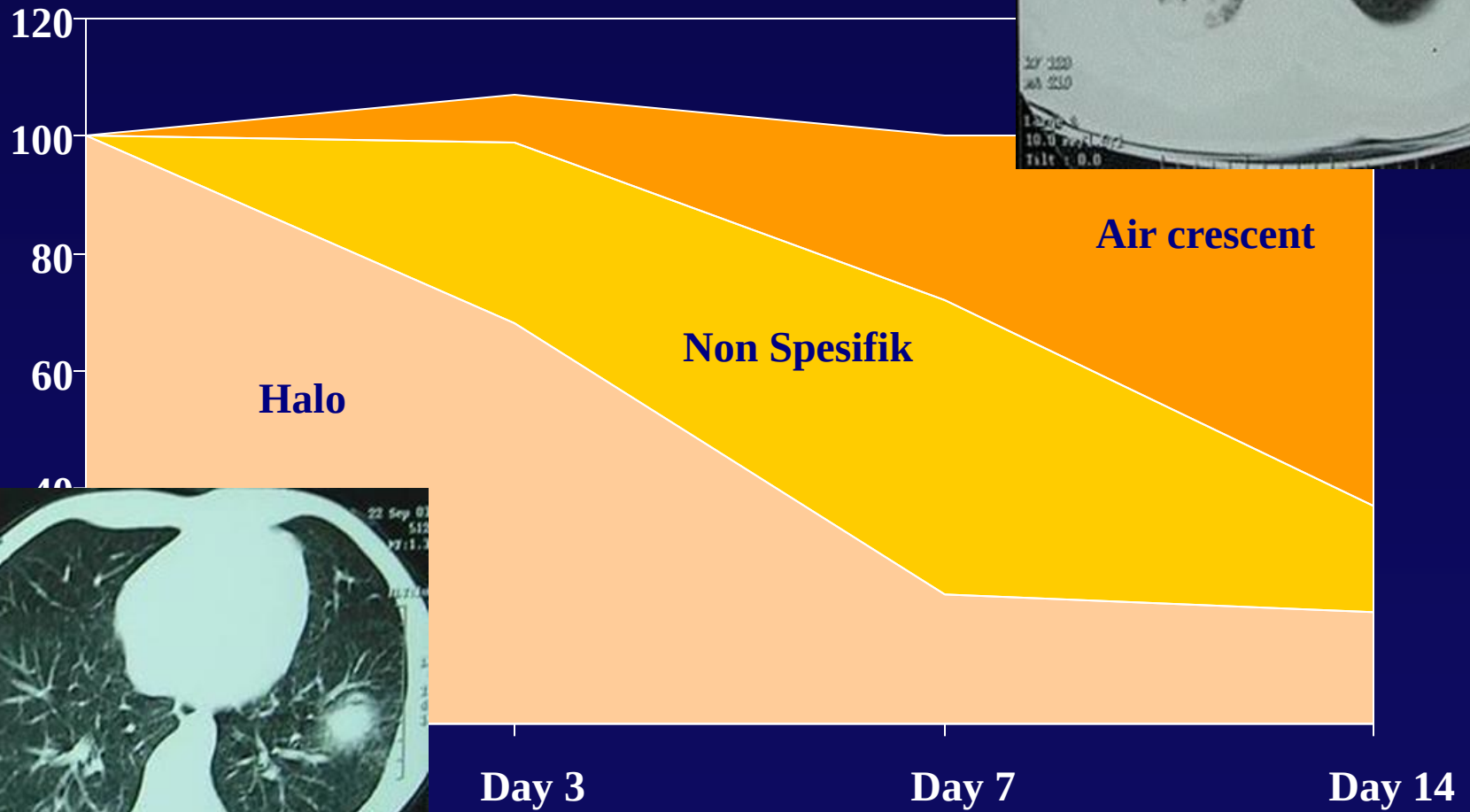
Marmara Üniversitesi Tıp Fakültesi



İFİ – Diagnostik Yöntemler

Metod	Avantajlar	Dezavantajlar
Kültür	• Özgün • Altın standart	• İnvaziv işlem gerektirebilir • Yalancı negatif olabilir
Histopatoloji	• Etken direk olarak görülür • Steril dokuda etken gösterilirse IFI kanıtlanır	• İnvaziv işlem gerektirir • Spesifik tür tayini yapılamaz
HRCT	• Non – invaziv • Pulmoner enfeksiyon tayininde çok etkili	• Non - specific
Beta Glucan	• Yüksek NPV	• Yalancı pozitiflik • Zigomycetes negatif
Galactomannan	• Özgün test	• Yalancı pozitiflik • Yalancı negatiflik
PCR	• Özgün test	• Yalancı pozitiflik • Standardize edilemedi • Ticari formu yok

HRCT



CT scan evolution during IPA

Peripheral halo

triangular shape

Air-crescent sign

d0 - d5

d5 - d10

d10 - d20

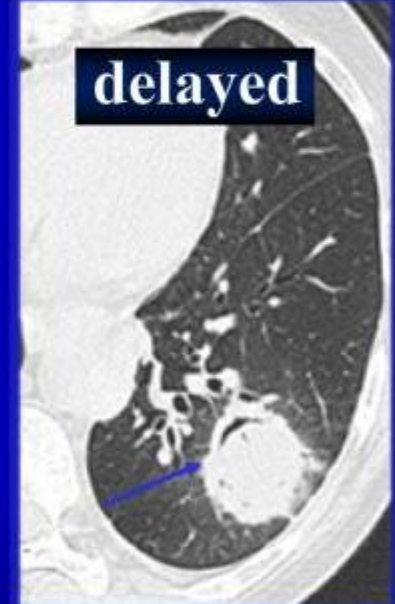
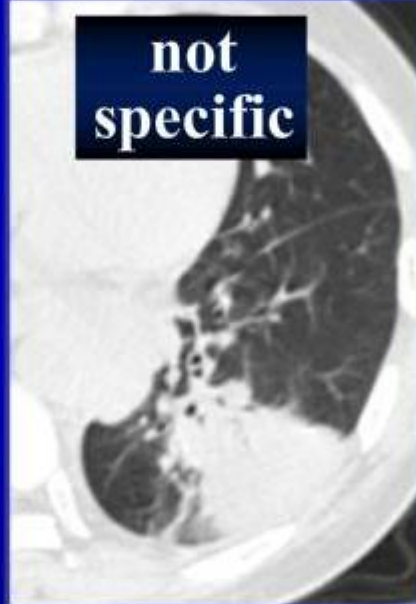
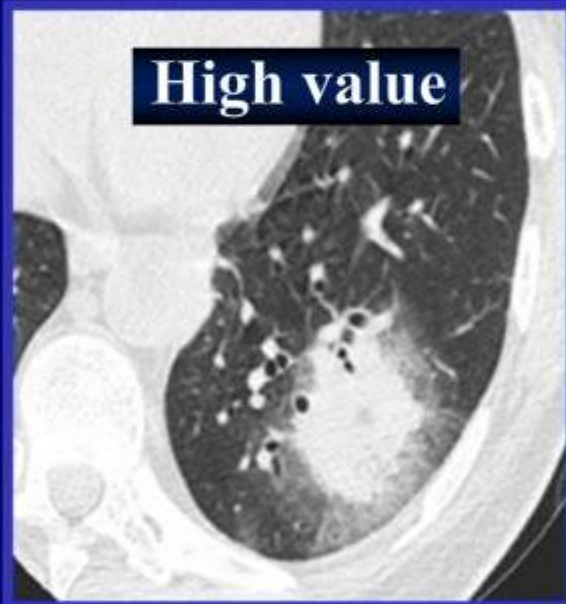
High value

**not
specific**

delayed

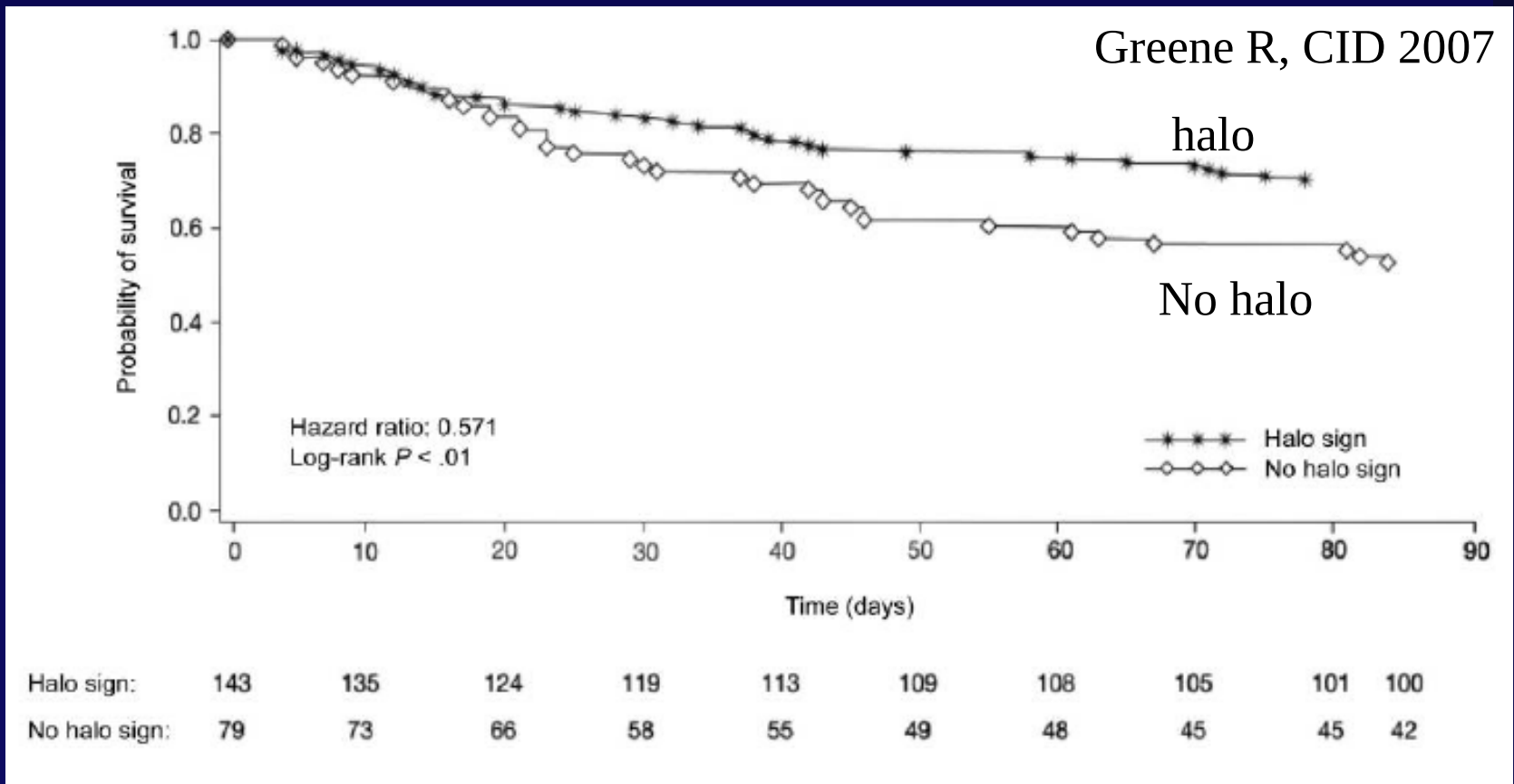
Neutropenia

PMN >> 500

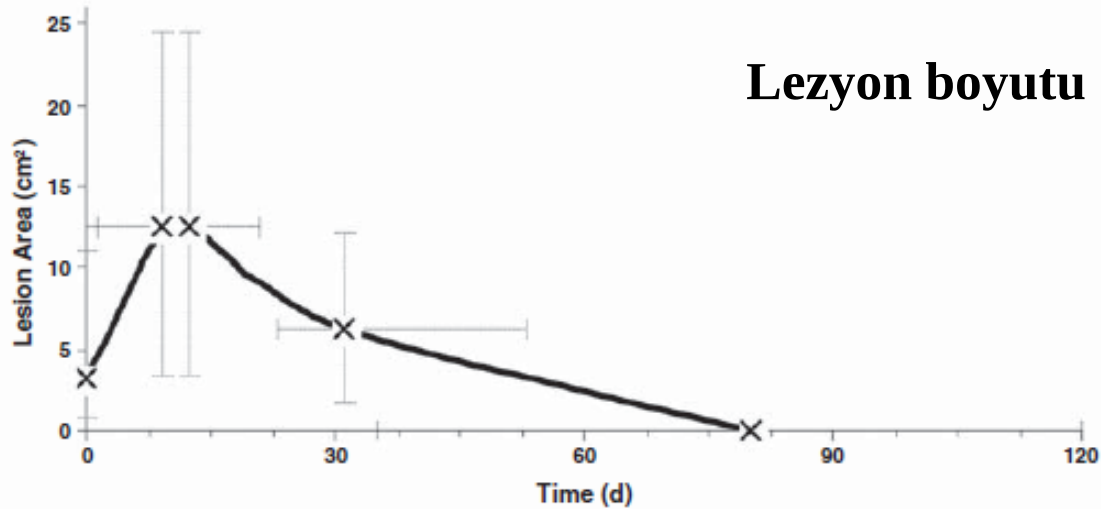


Halo İşareti ve Klinik Önemi

- Halo işareti varlığında antifungal tedavi başlanması mortaliteyi azaltabilir

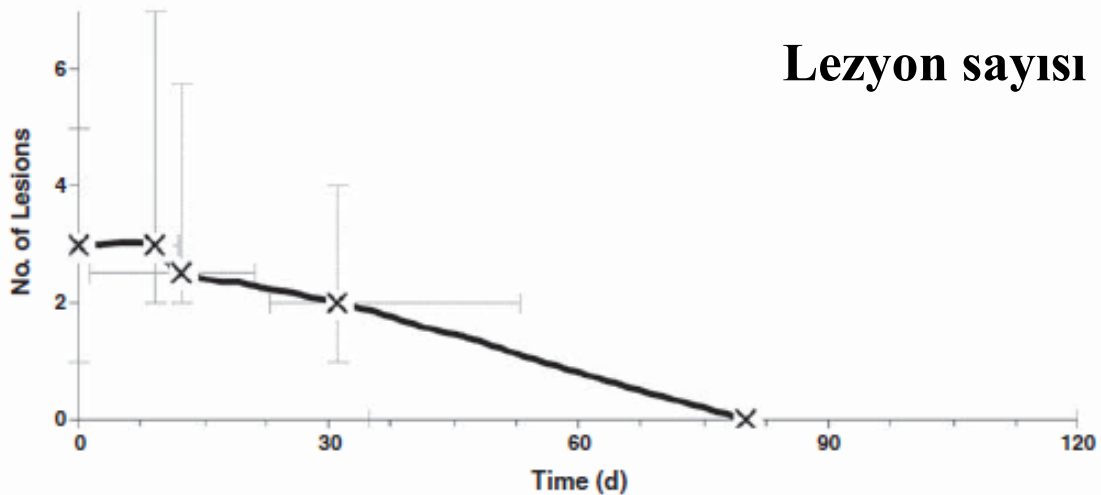


Lezyon boyutu



A

Lezyon sayısı



B

Fig. 1—Graphs illustrate CT kinetics.

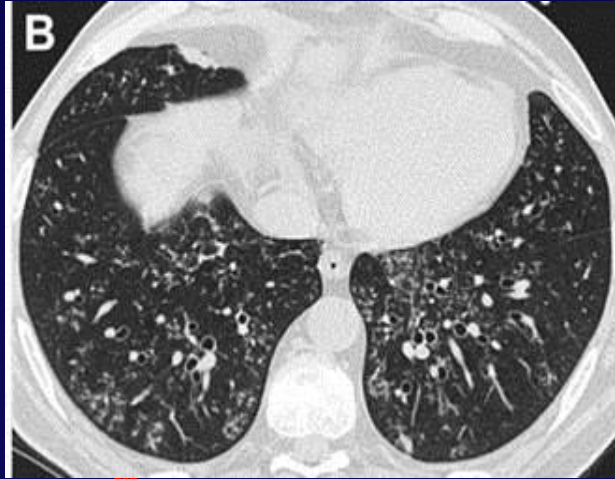
A and B, Kinetics of lesion size (A) and numbers (B). Median values and interquartile ranges are provided for lesion size, lesion number, and time (days). Time points (X) indicated are first diagnosis, first sight of maximal area, last sight of maximal area, time at halved maximal area, and time at complete radiologic remission. Mean values for these time points were 0, 9, 16, and 85.5 days, respectively. Ninety percent of 40 patients showed increase of lesion size after day of diagnosis; 62.5% had reduction down to 50% of maximum size, and 42.5% had complete radiologic remission.

Nodül kinetiği

Brodefel et al ,AJR 2006

Aspergillozun Farklı Hastalıklarda Prezantasyonu

Hava yolu invaziv



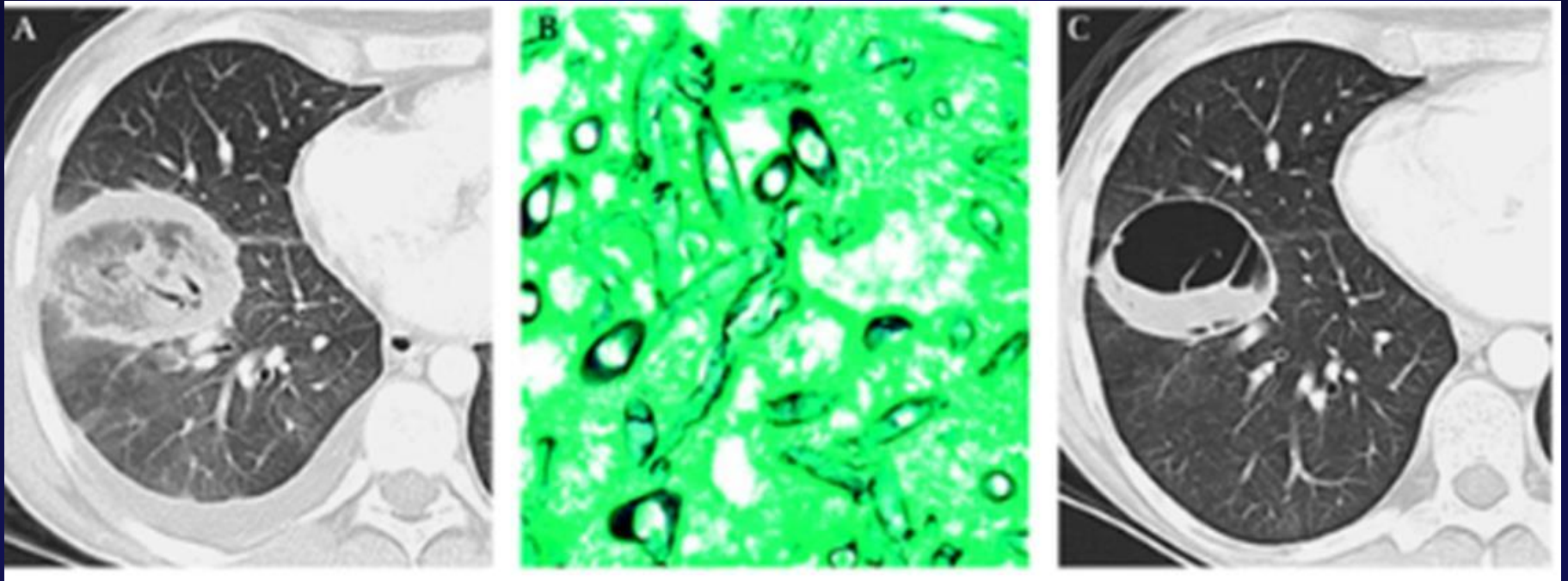
Anjiyo - invaziv

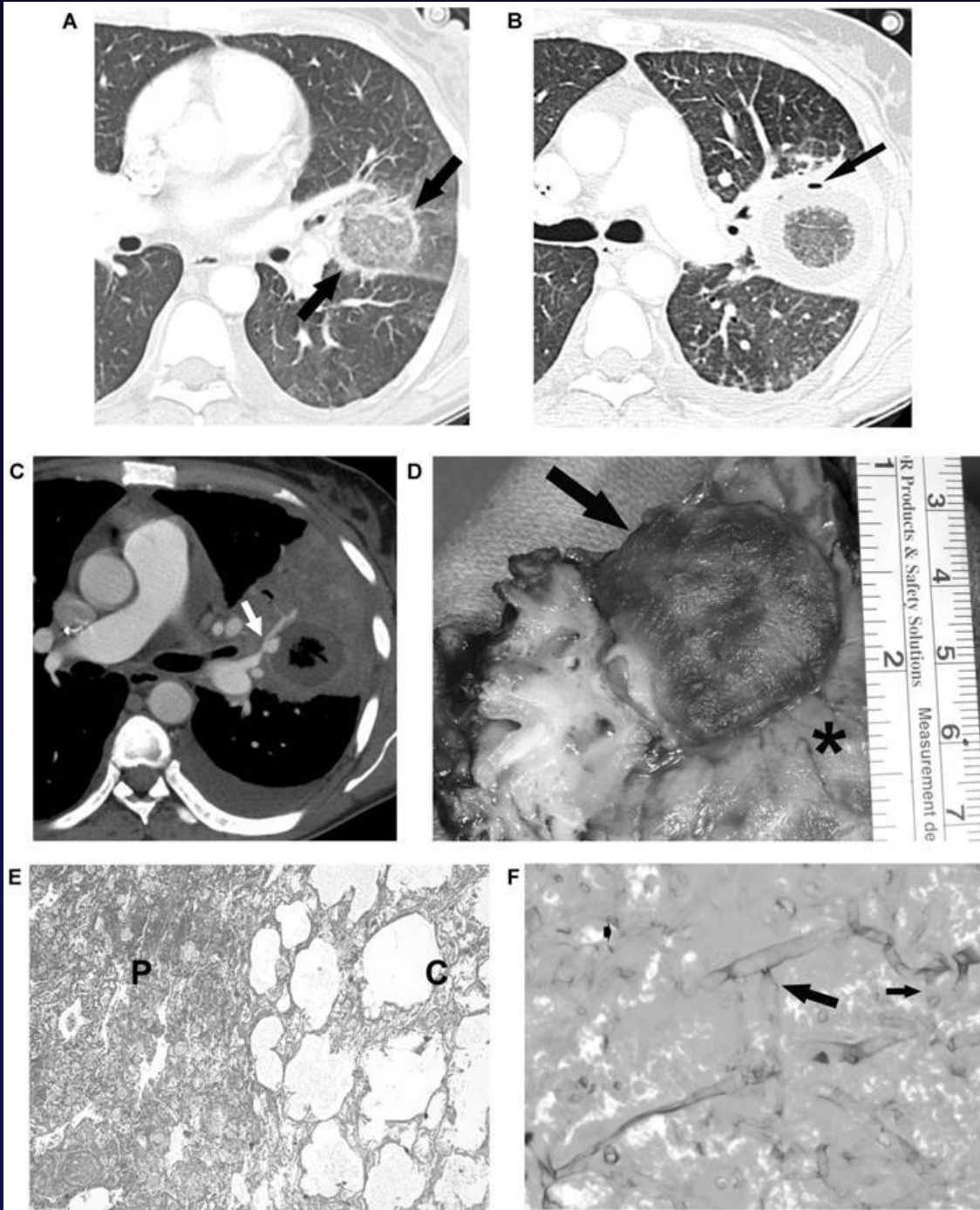


Bulgu	HSCT (n=23)	Akut Lösemiler (n=22)
Anjiyo-invaziv	3 (13%)	10 (45%)
Havayolu invaziv	10 (44%)	3 (14%)

Ters Halo İşareti (Reversed halo)

- İmmümkompromize hastaların %4 'ünde görülür
- Erken dönem zigomikoz





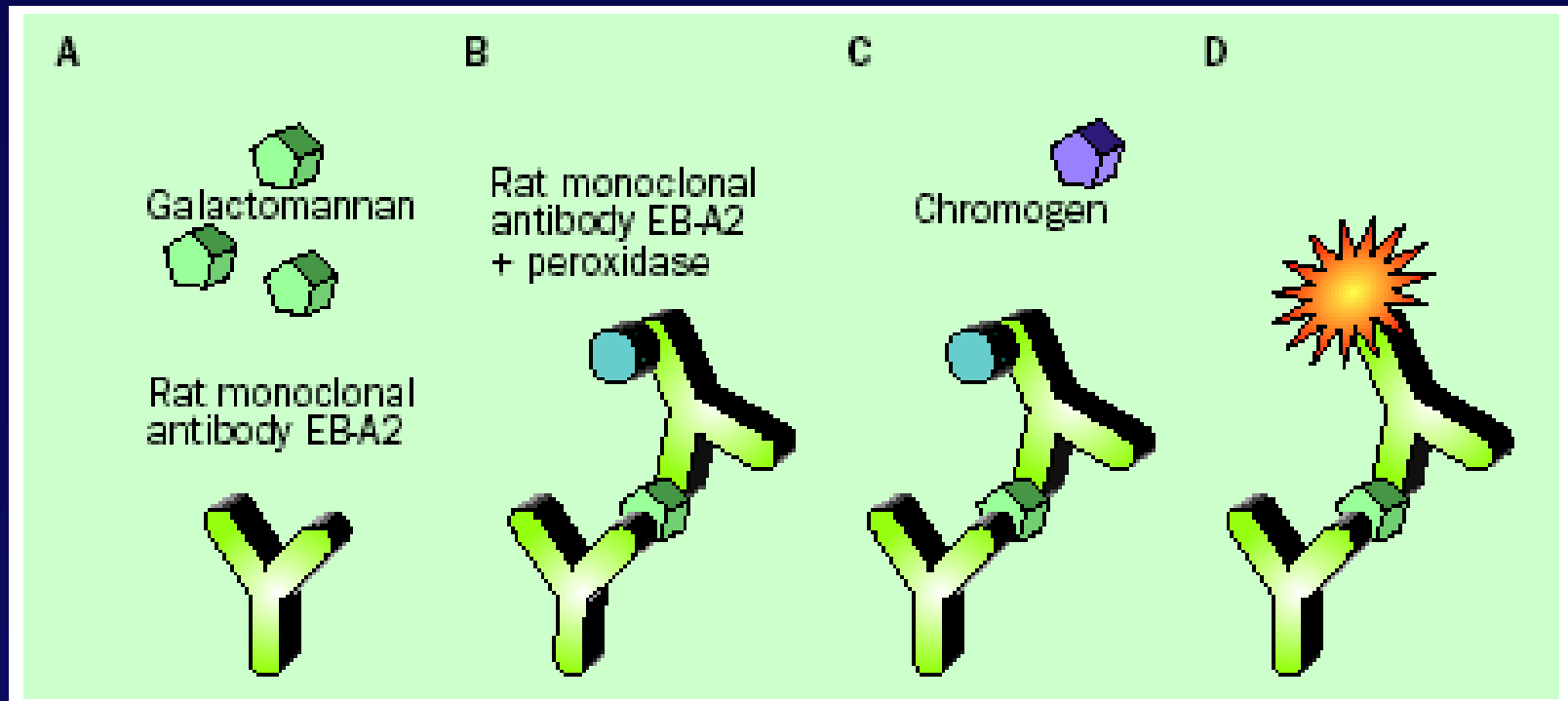
Ters Halo:

- BOOP
- Zigomikoz
- Aspergilloz (nadir)

Wahba H, CID, 2008,

GALAKTOMANNAN TESTİ

Double sandwich - ELISA yöntemi



GALAKTOMANNAN TESTİ

- İnvazif aspergillus infeksiyonlarında
 - Duyarlılık %33 – 94
 - Özgüllük %75 – 100
 - Yalancı pozitiflik %6 –14 (yetişkinlerde)
 - Pediatrik vakalarda %83

Marr, K. Clin Infect Dis 2005

Cut – off GM index değerleri

- **Ardışık 0.5 ve üzerinde iki pozitiflik**
- **≥ 0.7 tek bir değer**

Cut-off	studies	N	sensitivity	95%CI	specificity	95%CI
0.5	7	901	0.78	0.61-0.89	0.81	0.72-0.88
1	12	1744	0.75	0.59-0.86	0.91	0.84-0.95
1.5	17	2600	0.64	0.5-0.77	0.95	0.91-0.97

GM Cut – off index değerleri

İnsidans 8% 100 vaka		
	IFD vaka = 8	IFD olmayan = 92
Cut off	Yalancı negatif	Yalancı pozitif
0.5	2	17
1	2	8
1.5	3	5

Yalancı pozitiflik sebepleri

- Non-invazif enfeksiyona bağlı geçici antijenemi ?
- Diğer mantar ve bakterilerle çapraz reaksiyon
- Siklofosfamid
- Cotton swabs
- Mukozit sonrası barsaklardan translokasyon
- Galaktomannan içeren antibiyotikler
 - **Pip/tazo, Amox/Clav, Ampicillin**

Yalancı negatiflik sebepleri

- Geniş spektrumlu antifungal kullanımı
- Az miktarda anjiyo-invazyon olması
- Anti-aspergillus antikor varlığı
- Düşük mantar yükü
- Uygunsuz test tekniği, numune alımı, saklama koşulları

Galactomannan: **All** recommendations for a strategy in adults

- Prospective monitoring of serum is a feasible approach for adult neutropenic patients undergoing intensive chemotherapy for leukemia or receiving an allogeneic stem cell transplantation for the early diagnosis of invasive aspergillosis (All) [Note: Plasma may also be used (CIII)]
- Galactomannan monitoring is recommended every three to four days for patients admitted to hospital (All)
- Persistent galactomannan antigenaemia during therapy is a poor prognostic sign and should prompt a reassessment of the management of the patient (BII)
- A diagnostic driven strategy that incorporates galactomannan monitoring should be combined with high resolution CT imaging, appropriate clinical and microbiological evaluation to diagnose invasive aspergillosis early. A single sample with a galactomannan index of ≥ 0.7 or 2 consecutive samples with an index of ≥ 0.5 should prompt a thorough diagnostic work-up (All)

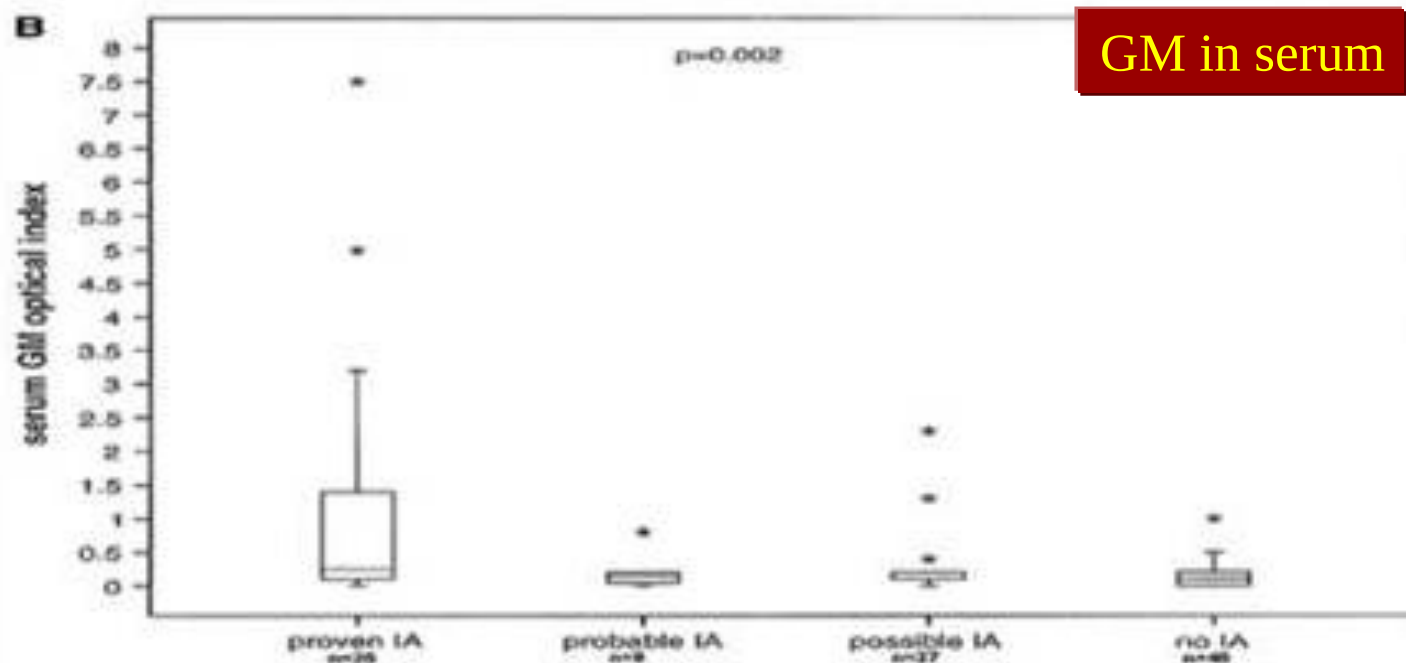
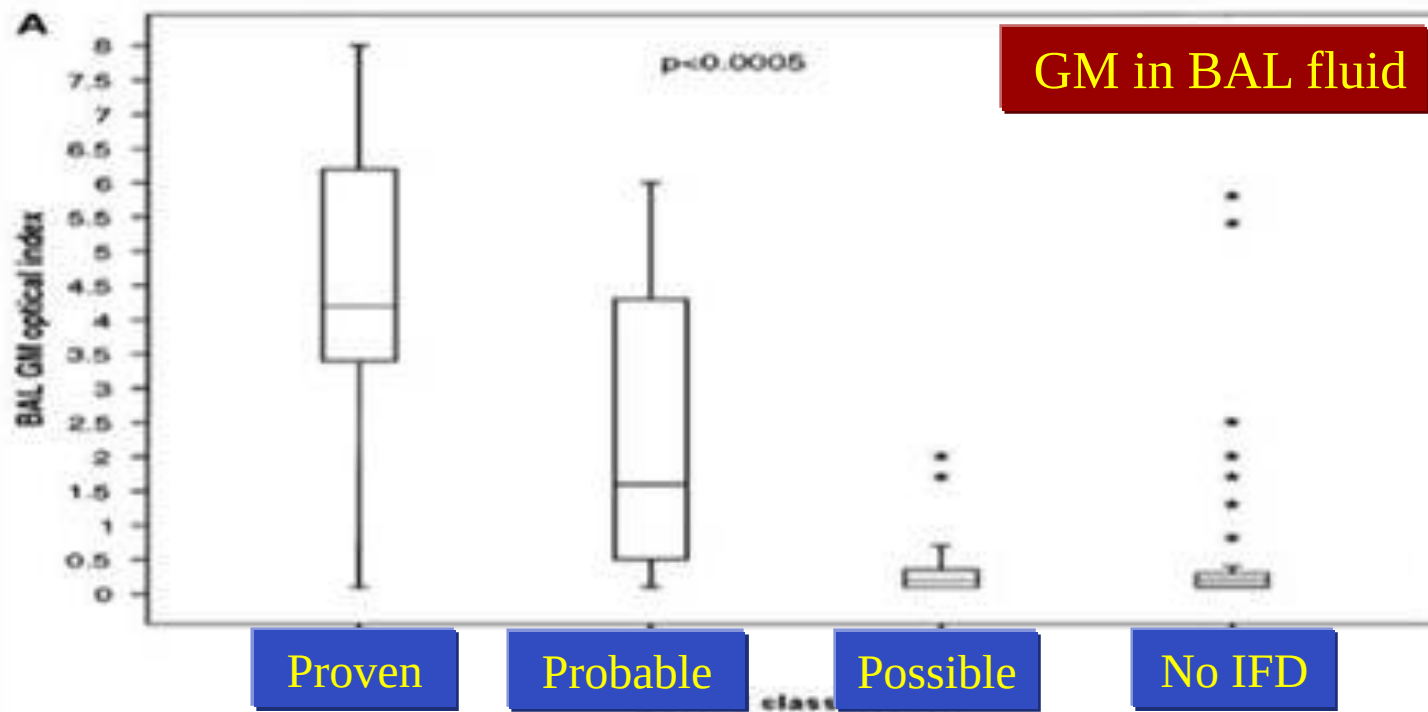


Galactomannan in Bronchoalveolar Lavage Fluid

A Tool for Diagnosing Aspergillosis in Intensive Care Unit Patients

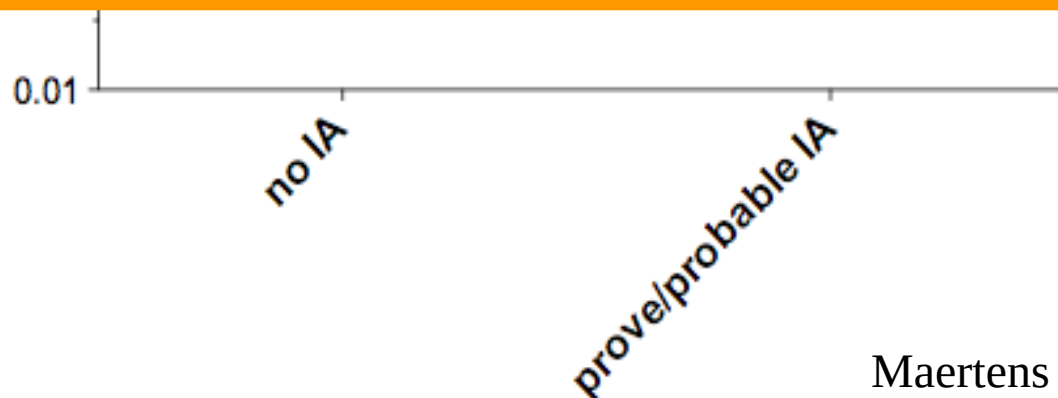
Wouter Meersseman¹, Katrien Lagrou², Johan Maertens³, Alexander Wilmer¹, Greet Hermans¹, Steven Vanderschueren¹, Isabel Spriet⁴, Eric Verbeken⁵, and Eric Van Wijngaerden¹

Am J Respir Crit Care Med Vol 177. pp 27–34, 2008

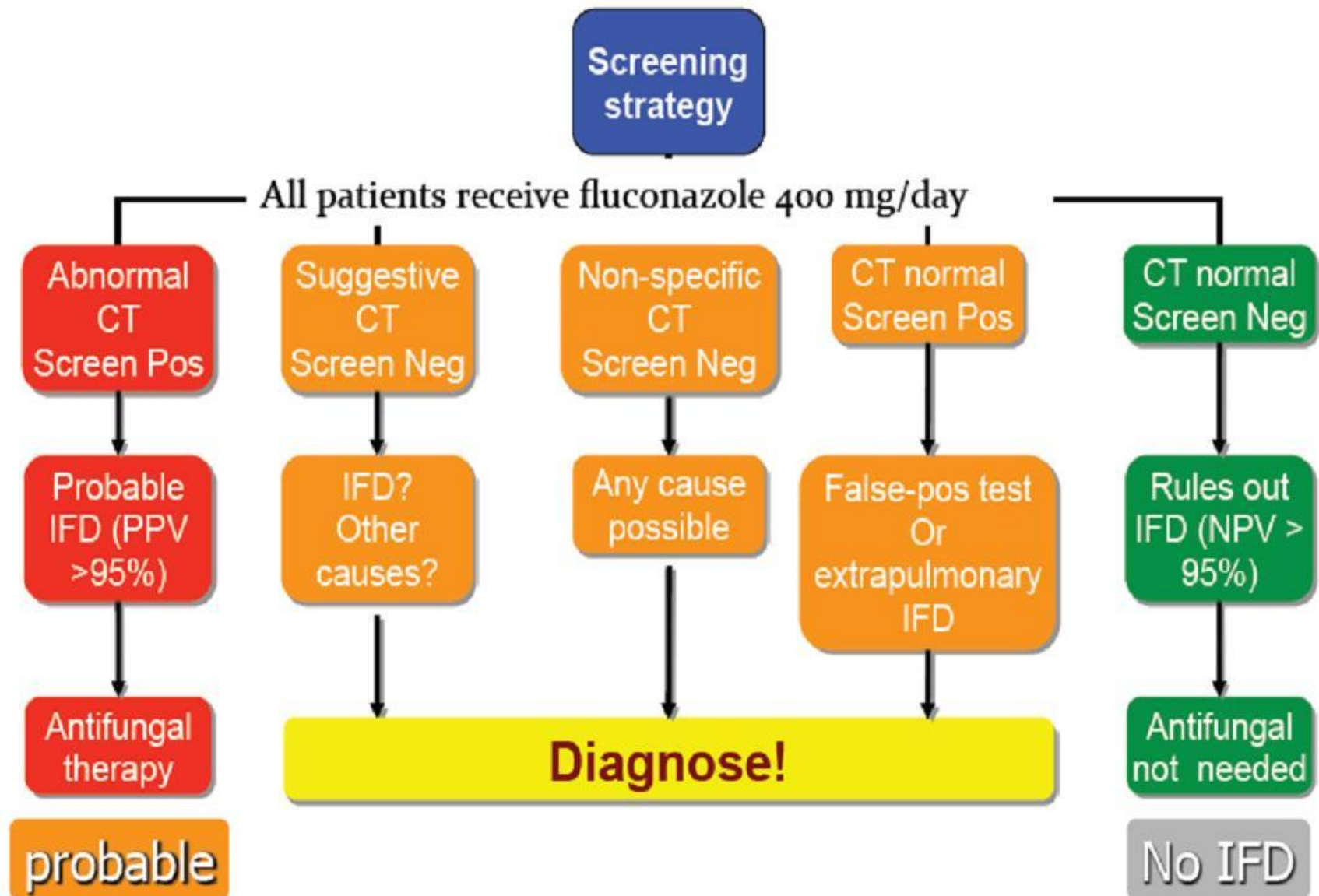


Hematolojik Maliniteli hastalarda BAL GM Düzeyi

Cut-off	Sensitivity (%)	95%CI	Specificity (%)	95%CI
0.5	96.5	91.9-100	80.4	68.4-92.6
1	91.3	84.2-98.6	87.7	77.8-97.8
1.5	84.4	75.2-93.8	90.2	81.2-99.3



Galactomannan and CT-scan strategy

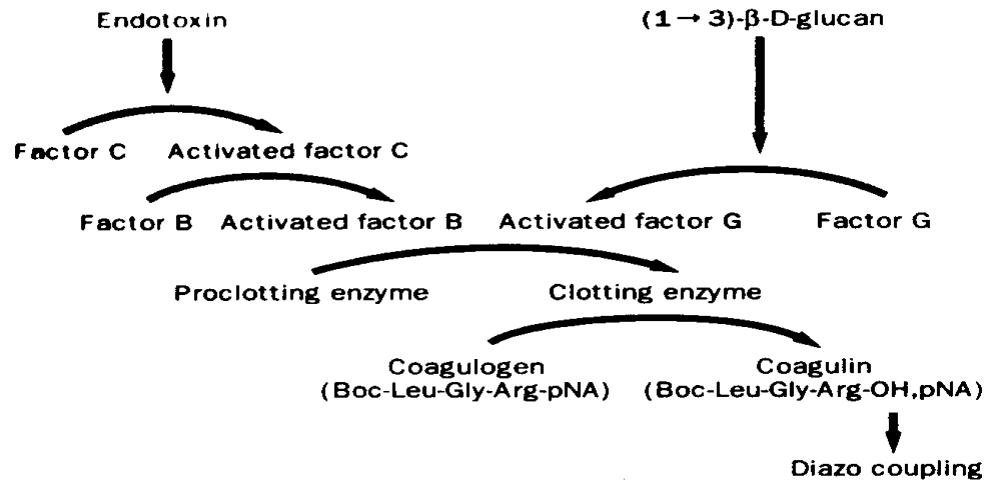


Beta Glukan Testi



Horseshoe Crab
amebosit lizatından
elde edilen **Faktör G**
ile beta glukanın
reaksiyonu

Conventional limulus test



G-test

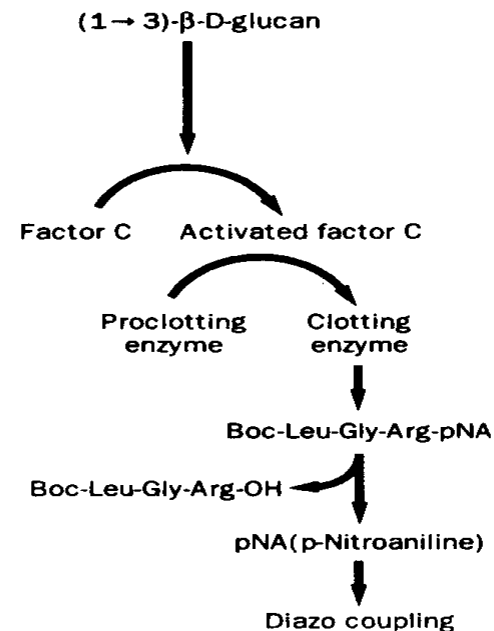


Figure 1: Principle of G test and conventional limulus test
Limulus test reacts to both endotoxin and (1→3)-β-D-glucan, whereas G test reacts to the glucan only.

(1,3)-Beta-D-Glucan Detection (Fungitell®)



İFi de FDA lisansı: May 21st, 2004



Tespit edilemeyenler :

- Zygomycetes
- Cryptococcus* spp



Test numunesi : 5µl serum

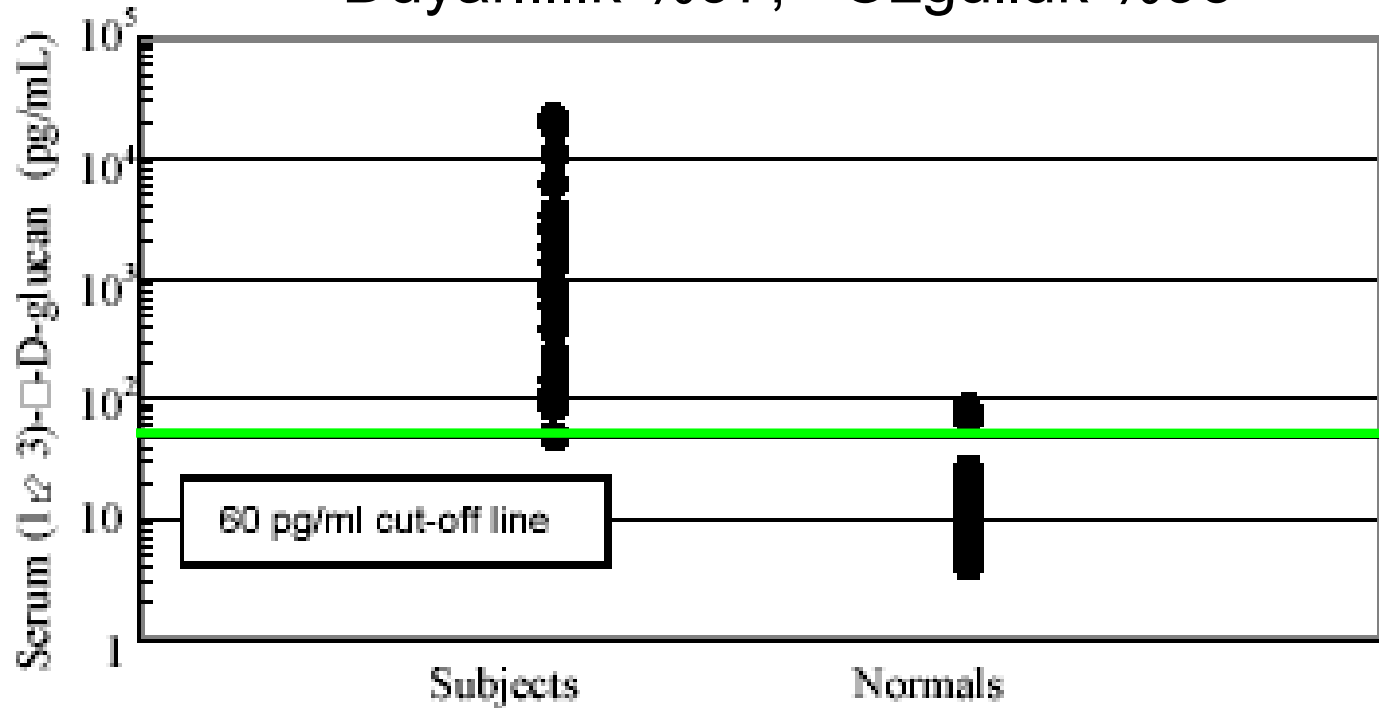


Test süresi: 2 saat

BETA GLUKAN TESTİ

Kandidemik 30 hastada B glukan testi

Duyarlılık %97, Özgüllük %93



Glucatell® (1.3)-Beta-DG

Nötropenik Hastalardac IFI Tanı Çalışması

- **283 AML hastası**
 - Tüm kemoterapi süresince haftada 2 serum örneği
 - Hasta başına ortalama 7.3 numune alındı
 - Total 2070 serum örneği
 - Hastalar itrakonazol veya kaspofungin profilaksisi altında
- **IFI tanısı EORTC/MSG kriterlerine göre yapıldı**
- **Beta glukan testleri kör olarak yapıldı**

Glucatell® (1.3)-Beta-DG

Nötropenik Hastalardac IFI Tanı Çalışması

- **15 Kanıtlanmış fungemi**
 - 6 *C. glabrata*
 - 3 *T. asahii*
 - 2 *C. krusei*
 - 1 *C. albicans*, *C. tropicalis*, *C. parapsilosis* & *Fusarium* sp
- **1 Kanıtlanmış fungal pnömoni: *A. terreus***
- **4 Yüksek olasılıklı fungal pnömoni**
 - 2 *A. fumigatus*
 - 1 *A. terreus* & *C. albicans*; 1 *Fusarium*
- **33 Düşük olasılıklı fungal pnömoni**

Glucate® (1.3)-Beta-DG

Beta Glukan ⊕ serum örneği sayısı	Kanıtlanmış veya Yüksek Olasılıklı IFI			
	% S	% Sp	% PPV	% NPV

Glucate® (1.3)-Beta-DG

Beta Glukan ⊕ serum örneği sayısı	Kanıtlanmış veya Yüksek Olasılıklı IFI			
	% S	% Sp	% PPV	% NPV
1 ⊕ örnek	100	90	43	100

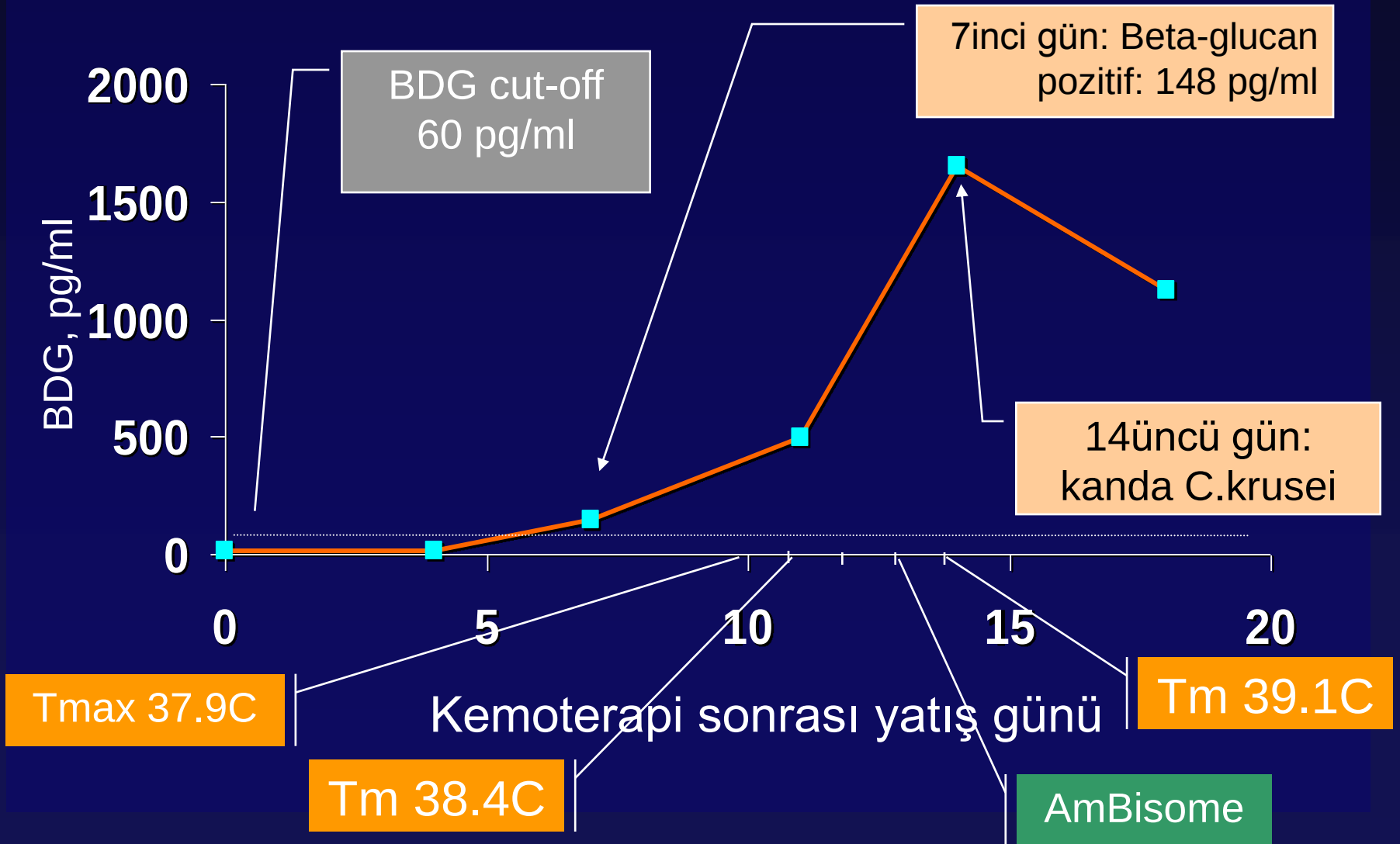
Glucate® (1.3)-Beta-DG

Beta Glukan $\oplus$ serum örneği sayısı	Kanıtlanmış veya Yüksek Olasılıklı IFI			
	% S	% Sp	% PPV	% NPV
1 $\oplus$ örnek	100	90	43	100
≥ 2 $\oplus$ örnek	65	96 	57	97

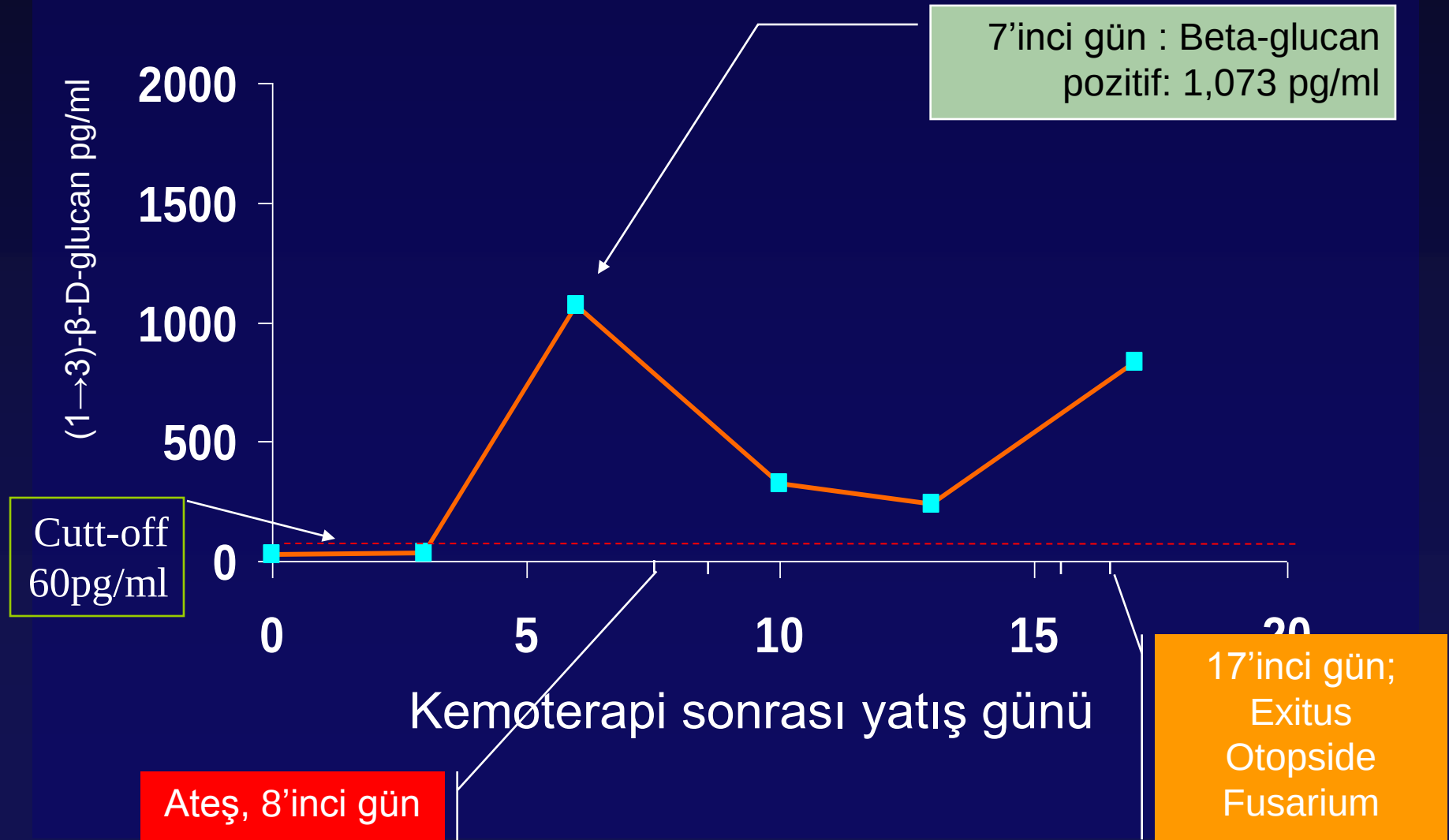
Glucate® (1.3)-Beta-DG

Beta Glukan ⊕ serum örneği sayısı	Kanıtlanmış veya Yüksek Olasılıklı IFI			
	% S	% Sp	% PPV	% NPV
1 ⊕ result	100	90	43	100
≥ 2 ⊕ results	65	96 ↑	57	97
≥ 3 ⊕ örnek	60	99 ↑	80	96

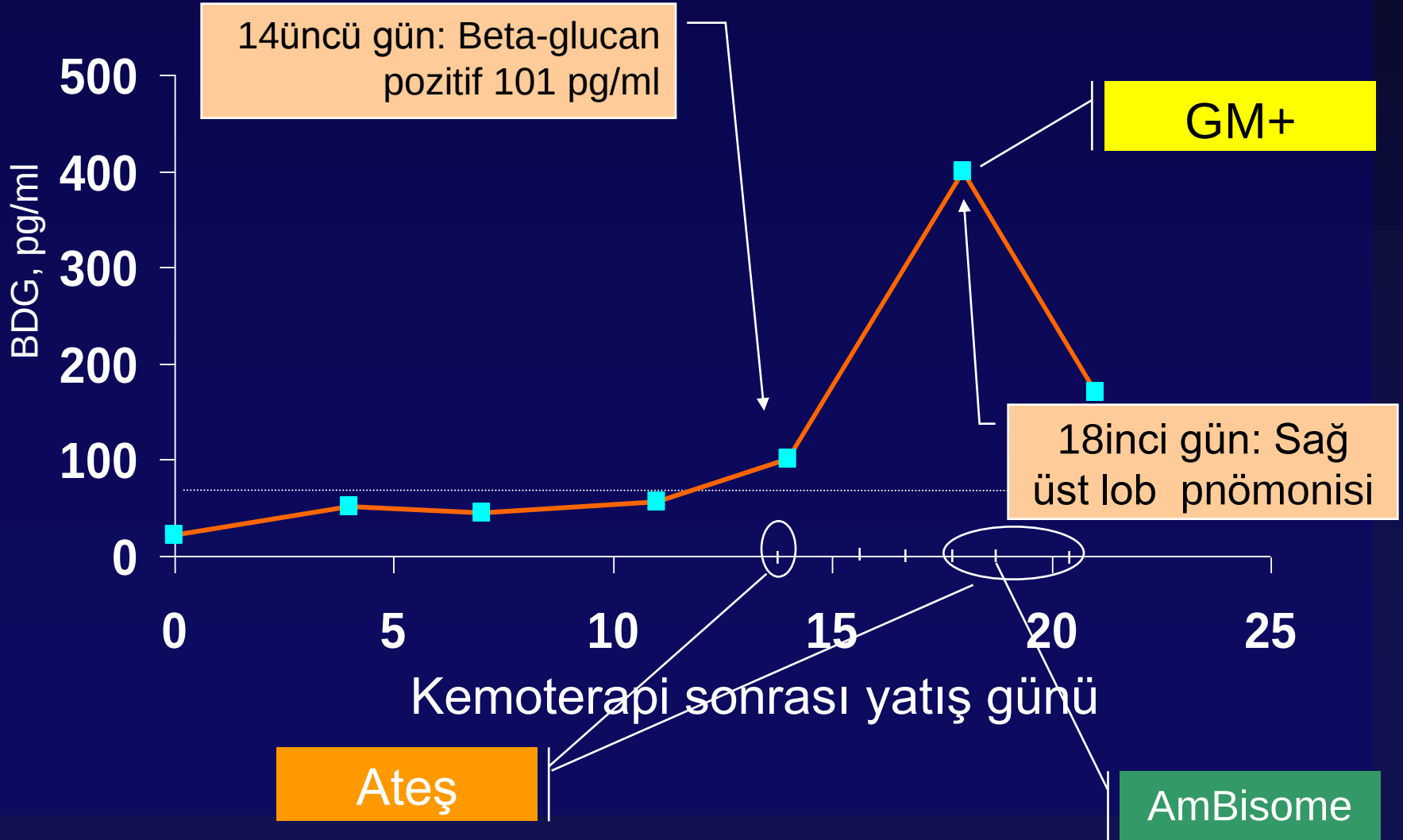
Kanıtlanmış Fungemi Vakası ve BG testi



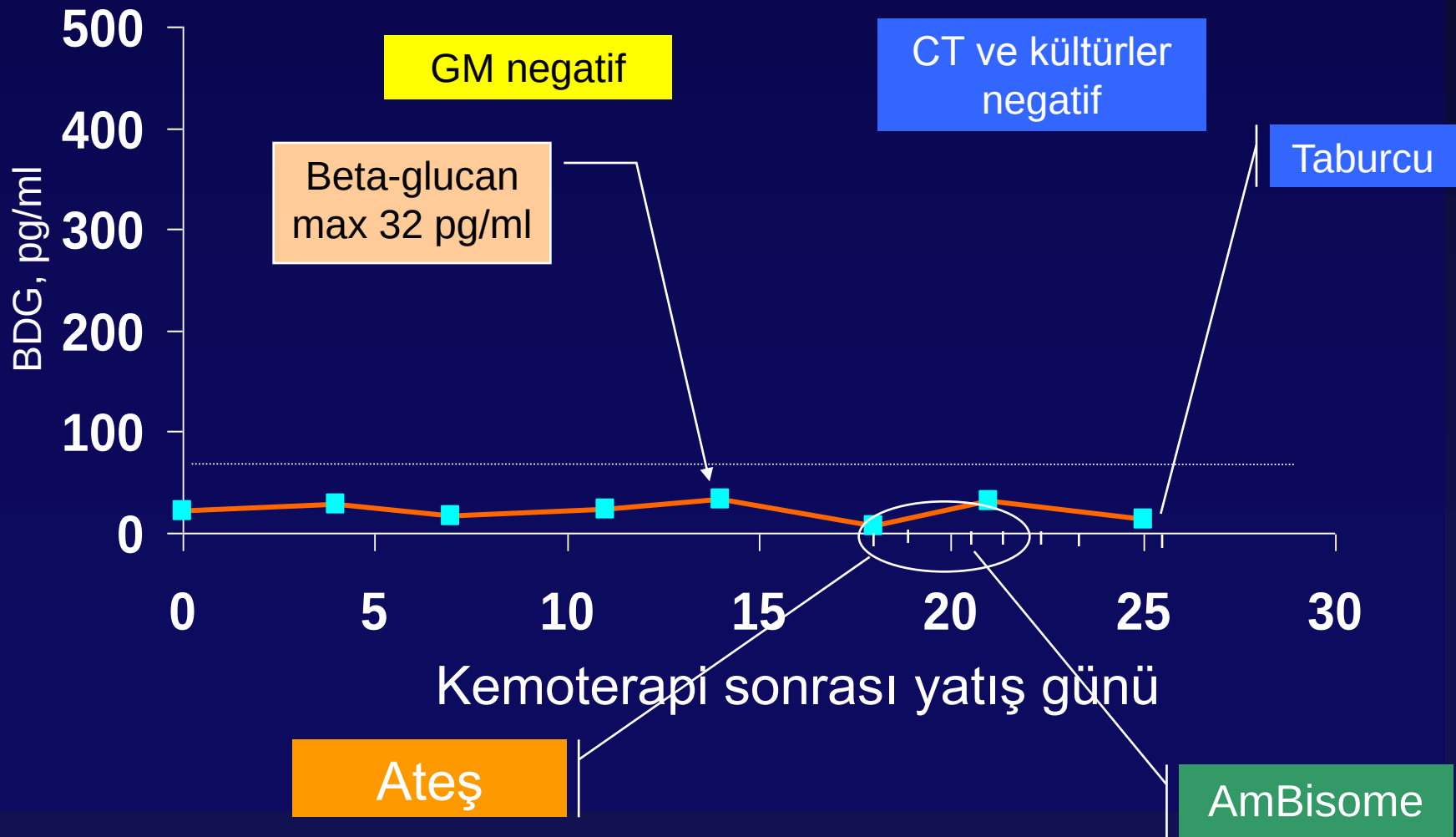
Kanıtlanmış Fungemi Vakası ve BG testi



Düşük İhtimalli Fungal Pnömoni Vakası ve BG Testi



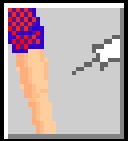
Febril Nötropenik FUO vakası ve Beta Glucan Testi



Yalancı Pozitiflik Sebepleri (literatür bilgisi)



- Cerrahi tamponlar, gazlı bez: Nakao et al' 97
 - Cerrahi YBÜ de erken dönemde %30 yalancı pozitiflik
 - Ostrosky Zeichner L, ICAAC 2005, M-168



- İmmünglobulin ürünleri: Ikemura et al' 89



- Selüloz hemodiyaliz membranları: Kato et al' 01

Contribution of (1→3)- β -D-Glucan Chromogenic Assay to Diagnosis and Therapeutic Monitoring of Invasive Aspergillosis in Neutropenic Adult Patients: a Comparison with Serial Screening for Circulating Galactomannan

Carmen Pazos,^{1*} José Pontón,² and Amalia Del Palacio¹

*Unidad de Micología, Departamento de Microbiología, Hospital Universitario 12 de Octubre, Madrid,¹ and
Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología,
Universidad del País Vasco, Bilbao, Vizcaya,² Spain*

Kanıtlanmış / Yüksek olasılıklı İnvazif Aspergilloziste **Galaktomannan** ve **Beta Glukan**

	Duyarlılık	Özgüllük	PPV	NPV
Beta Glukan	88	90	70	96
Galaktomannan	88	90	70	96
Kombine Analiz*	88	100	100	96

***Beta glukan daha erken pozitifleşiyor**

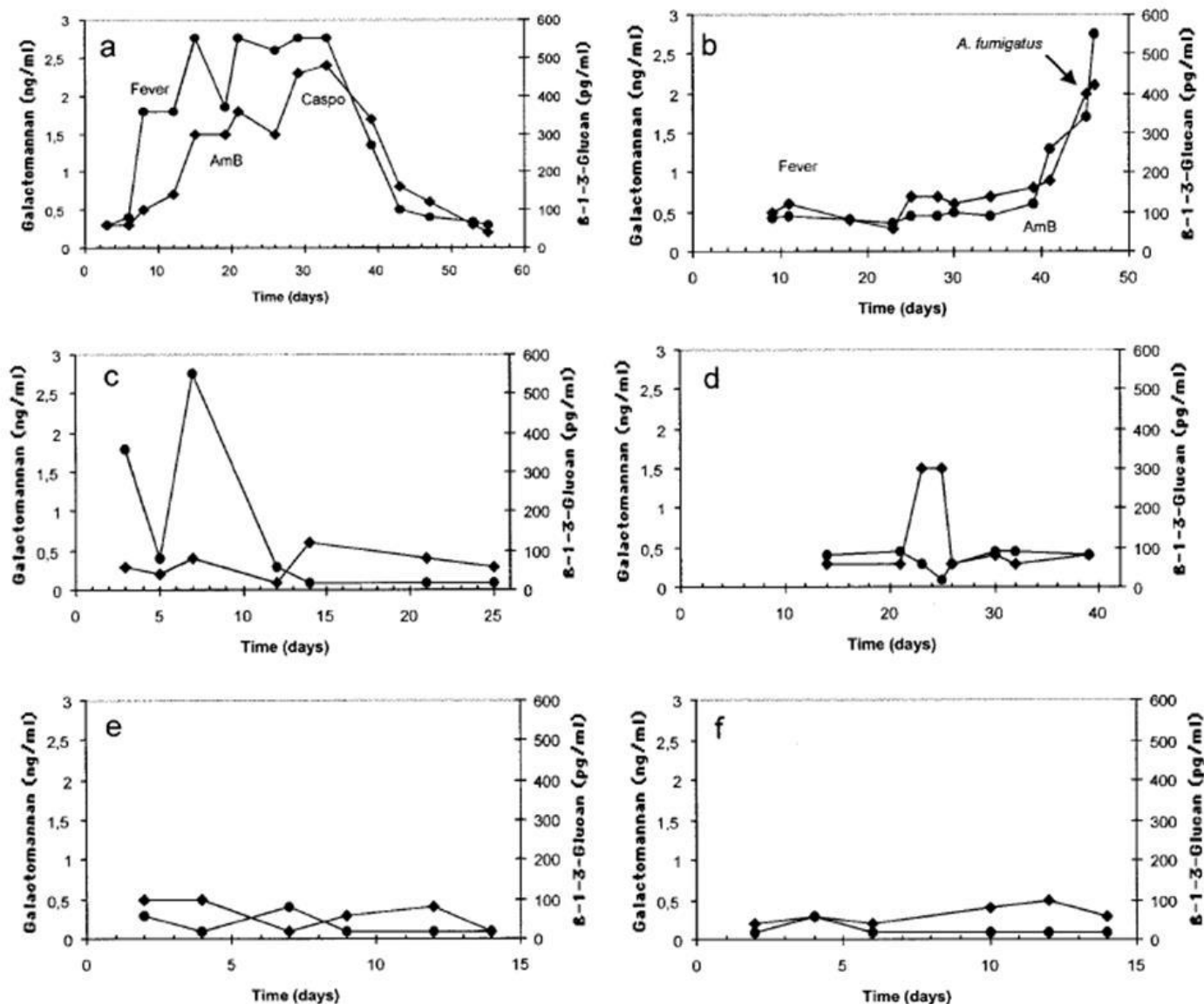


FIG. 1. Representative kinetics of BG (●) and GM (◆) in different patients. (a) Proven IA in a patient with acute myeloid leukemia who responded to treatment with amphotericin B and caspofungin. (b) Proven IA in a patient with chronic lymphocytic leukemia who did not respond to treatment with amphotericin B. (c) False-positive BG results in a patient with multiple myeloma and no IA. (d) False-positive GM results in a patient with non-Hodgkin's lymphoma and no IA. (e) Negative BG and GM results in a patient with acute myeloid leukemia and no IA who was colonized by *C. albicans* and *C. glabrata*. (f) Negative BG and GM results in a patient with chronic lymphocytic leukemia and no IA who was colonized by *C. albicans*. Abbreviations: AmB, amphotericin B; Caspo, caspofungin.

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0095-1137/09/\$08.00+0 doi:10.1128/JCM.00506-08
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Vol. 47, No. 1

Utility of Galactomannan Enzyme Immunoassay and (1,3) β -D-Glucan in Diagnosis of Invasive Fungal Infections: Low Sensitivity for *Aspergillus fumigatus* Infection in Hematologic Malignancy Patients[▽]

R. Y. Hachem,* D. P. Kontoyiannis, R. F. Chemaly, Y. Jiang, R. Reitzel, and I. Raad

The Department of Infectious Diseases, Infection Control and Employee Health, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

TABLE 3. Performances of GM enzyme immunoassay and BG test for patients infected with different organisms (per sample)

Test and organism	Sensitivity (%)	Specificity (%)	PPV (%) ^a	NPV (%) ^a
GM enzyme immunoassay				
<i>A. fumigatus</i> (n = 69)	13	99	90	66
Non- <i>fumigatus</i> <i>Aspergillus</i> species (n = 39)	49	99	95	86
Other mold (n = 77)	6	99	83	62
BG test				
<i>A. fumigatus</i> (n = 69)	61	88	75	79
Non- <i>fumigatus</i> <i>Aspergillus</i> species (n = 39)	64	88	64	88
Other mold (n = 76)	47	88	72	72

^a PPV, positive predictive value; NPV, negative predictive value.

Diagnostic Accuracy of Serum 1,3- β -D-Glucan for *Pneumocystis jiroveci* Pneumonia, Invasive Candidiasis, and Invasive Aspergillosis: Systematic Review and Meta-Analysis

Akira Onishi,^a Daisuke Sugiyama,^a Yoshinori Kogata,^a Jun Saegusa,^a Takeshi Sugimoto,^b Seiji Kawano,^b Akio Morinobu,^b Kunihiro Nishimura,^{a,c} and Shunichi Kumagai^{a,d}

Departments of Evidence Based Laboratory Medicine^a and Clinical Pathology and Immunology,^b Kobe University Graduate School of Medicine, Kobe, Japan; Division of Evidence-Based Medicine and Risk Analysis, National Cerebral and Cardiovascular Center, Osaka, Japan^c; and Department of Rheumatic Diseases, Shinko Hospital, Kobe, Japan^d

	Sensitivite (%)	Spesifisite (%)	Heterojenite
Pneumocystis jiroveci	96	84	yok
IFI	80	82	belirgin

β -D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis

Drosos E. Karageorgopoulos,^{1,2} Evridiki K. Vouloumanou,¹ Fotinie Ntziora,^{1,2} Argyris Michalopoulos,^{1,3} Petros I. Rafailidis,^{1,4} and Matthew E. Falagas^{1,4,5}

¹Alfa Institute of Biomedical Sciences; ²Department of Medicine, Laikon General Hospital, and ³Intensive Care Unit and ⁴Department of Medicine, Henry Dunant Hospital, Athens, Greece; and ⁵Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts

We aimed to assess the accuracy of measuring serum or plasma (1 $\rightarrow$ 3)- β -D-glucan (BDG) for the diagnosis of invasive fungal infections (IFIs) by means of a meta-analysis of relevant studies. We searched in bibliographic databases for relevant cohort or case-control studies. We primarily compared BDG between patients with proven or probable IFIs (excluding *Pneumocystis jirovecii* infections), according to the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group or similar criteria, and patients without IFIs (excluding healthy individuals as controls). A total of 2979 patients (594 with proven or probable IFIs), included in 16 studies, were analyzed. The pooled sensitivity of BDG was 76.8% (95% confidence interval [CI], 67.1%–84.3%), and the specificity was 85.3% (95% CI, 79.6%–89.7%). The area under the summary receiver operating characteristic curve was 0.89. Marked statistical heterogeneity was noted. BDG has good diagnostic accuracy for distinguishing proven or probable IFIs from no IFIs. It can be useful in clinical practice, if implemented in the proper setting and interpreted after consideration of its limitations.

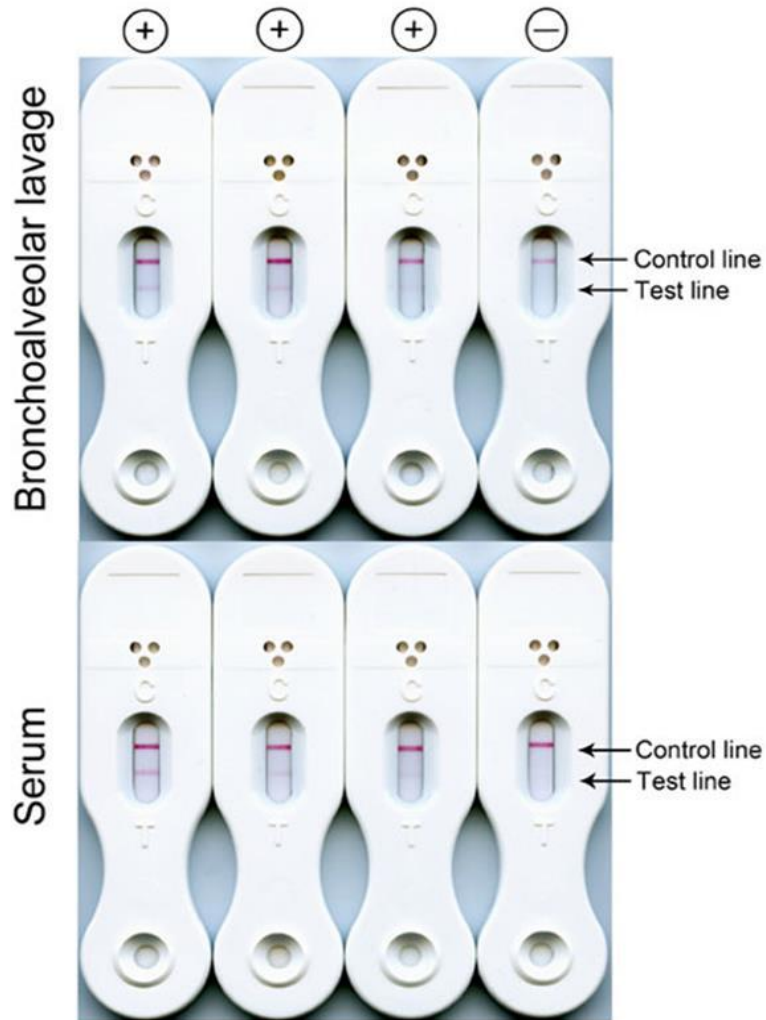
β -Glucan Antigenemia Assay for the Diagnosis of Invasive Fungal Infections in Patients With Hematological Malignancies: A Systematic Review and Meta-Analysis of Cohort Studies From the Third European Conference on Infections in Leukemia (ECIL-3)

Frédéric Lamothe,^{1,a} Mario Cruciani,^{2,a} Carlo Mengoli,³ Elio Castagnola,⁴ Olivier Lortholary,^{5,6,7} Malcolm Richardson,⁸ and Oscar Marchetti,¹ on behalf of the Third European Conference on Infections in Leukemia (ECIL-3)

ECIL 3: BII öneri, 2 kez bakılması

¹Université Paris Descartes, Service des Maladies Infectieuses et Tropicales, Hôpital Necker-Enfants Malades, Centre d'Infectiologie Necker-Pasteur, Paris; ²Institut Pasteur, Centre National de Référence Mycologie et Antifongiques, Unité de Mycologie Moléculaire, Paris; ³CNRS URA3012, Paris, France; and ⁴Mycology Reference Centre, Education and Research Centre, University Hospital of South Manchester (Wythenshawe Hospital), School of Translational Medicine, Manchester Academic Health Science Centre, University of Manchester, United Kingdom

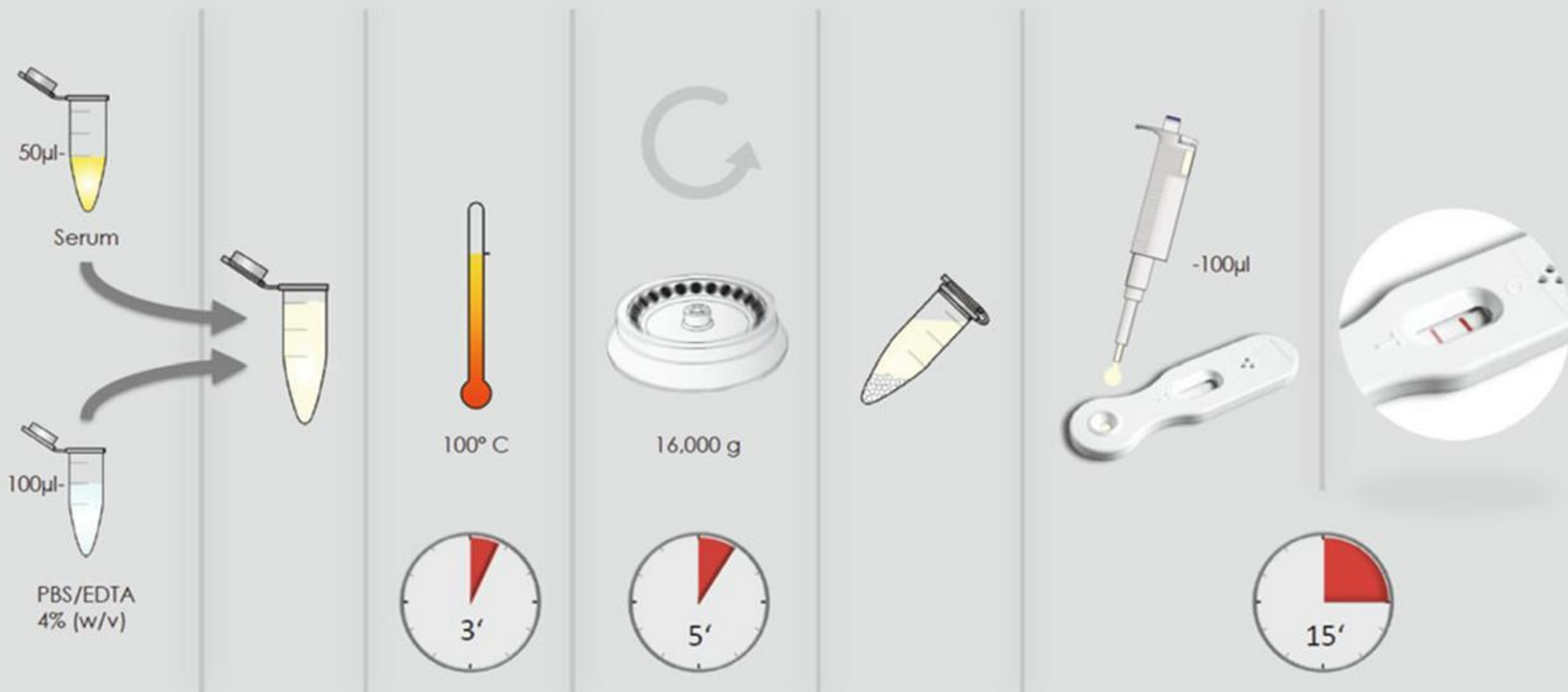
Aspergillus Lateral Flow Device (LFD)



Aspergillus LFD:

- Serum ve BAL
- 15 dakikada sonuç
- Muhtemel aspergillozda
 - %100 duyarlılık
 - %81 özgüllük
- *Candida* LFD
- Kriptokok LFD

- Monoclonal Antibody JF5: spesifik olarak Aspergillus α -1-3-, α 1-6-mannoprotein ile reaksiyon verir



Comparison of a novel *Aspergillus* lateral-flow device and the Platelia[®] galactomannan assay for the diagnosis of invasive aspergillosis following haematopoietic stem cell transplantation

J. Held • T. Schmidt • C. R. Thornton •
E. Kotter • H. Bertz

Criteria for positivity	Index test	TP	FP	FN	TN	Sensitivity	Specificity	PPV	NPV
One positive serum required	<i>Aspergillus</i> -LFD	4	12	6	79	40.0% (13.7-72.6)	86.8% (77.7-92.7)	25.0% (8.3-52.6)	92.9% (84.7-97.1)
	GM-EIA	4	10	6	81	40.0% (13.7-72.6)	89.0% (80.3-94.3)	28.6% (9.9-60.0)	93.1% (85.0-97.2)
Two consecutive positive sera required	<i>Aspergillus</i> -LFD	2	2	8	89	20.0% (3.5-55.8)	97.8% (91.5-99.6)	50.0% (9.2-90.8)	91.8% (83.9-96.1)
	GM-EIA	3	1	7	90	30.0% (8.1-64.6)	98.9% (93.2-99.9)	75.0% (21.9-98.7)	92.8% (85.2-96.8)

- Tek serum örneğinde pozitiflik: eşdeğer
- İkinci pozitiflik: GM üstün



ORIGINAL ARTICLE

ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients

O Marchetti^{1,6}, F Lamothe^{1,6}, M Mikulska², C Viscoli², P Verweij³ and S Bretagne^{4,5} and the European Conference on Infections in Leukemia (ECIL) Laboratory Working Groups⁷

¹Infectious Diseases Service, Department of Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland; ²Division of Infectious Diseases, San Martino University Hospital, Genoa, Italy; ³Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; ⁴Groupe Hospitalier Chenevier-Mondor, APHP, Laboratoire de Parasitologie-Mycologie, Créteil, France and ⁵Institut Pasteur, Unité de Mycologie Moléculaire, Centre National de Référence de Mycologie et des Antifongiques, Paris, France

Table 1 Grading system used for recommendations (based on IDSA-United States public health service grading)

<i>Strength of recommendation</i>	<i>Quality of evidence</i>
(A) Good evidence to support a recommendation for use	(I) Evidence from ≥ 1 properly randomized, controlled trial
(B) Moderate evidence to support a recommendation for use	(II) Evidence from ≥ 1 well-designed clinical trial, without randomization; from cohort or case-control analytical studies (preferably from > 1 center); from multiple case series; or from dramatic results from uncontrolled experiments
(C) Poor evidence to support a recommendation for use	(III) Evidence from opinions of respected authorities, based on clinical experience, descriptive studies or reports of expert committees

ECIL 3

	GM	Beta Glukan	Mannan - antimannan	Kriptokok antijeni	PCR
İnvaziv Aspergilloz	Evet	Evet			Evet
İnvaziv Kandidiyaz		Evet	Evet		
Kriptokokkoz				Evet	
EORTC/MSG İFi Tanısında	A II	B II	C II	A II	???

PCR klinik standardizasyon yeterli klinik validasyonu yok

TEŞEKKÜRLER