Neutrophils and Damage During Invasive Aspergillosis: Superoxide and *STAT3*

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Why study immune defects?

The names are cool

Nobody else knows about them

Rare (likely to be important)

Congenital or acquired

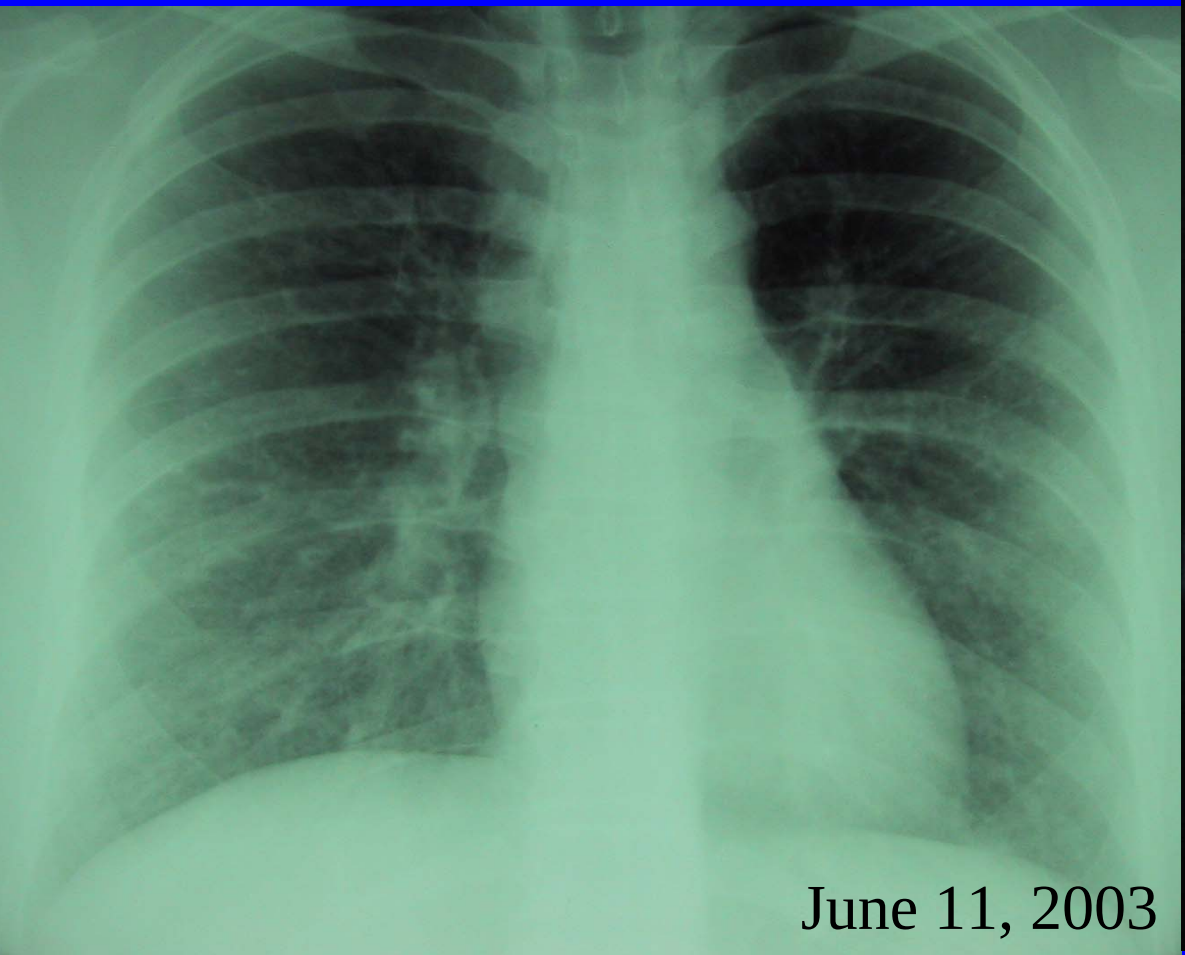
Genetic (usually single gene)

Informative about pathways

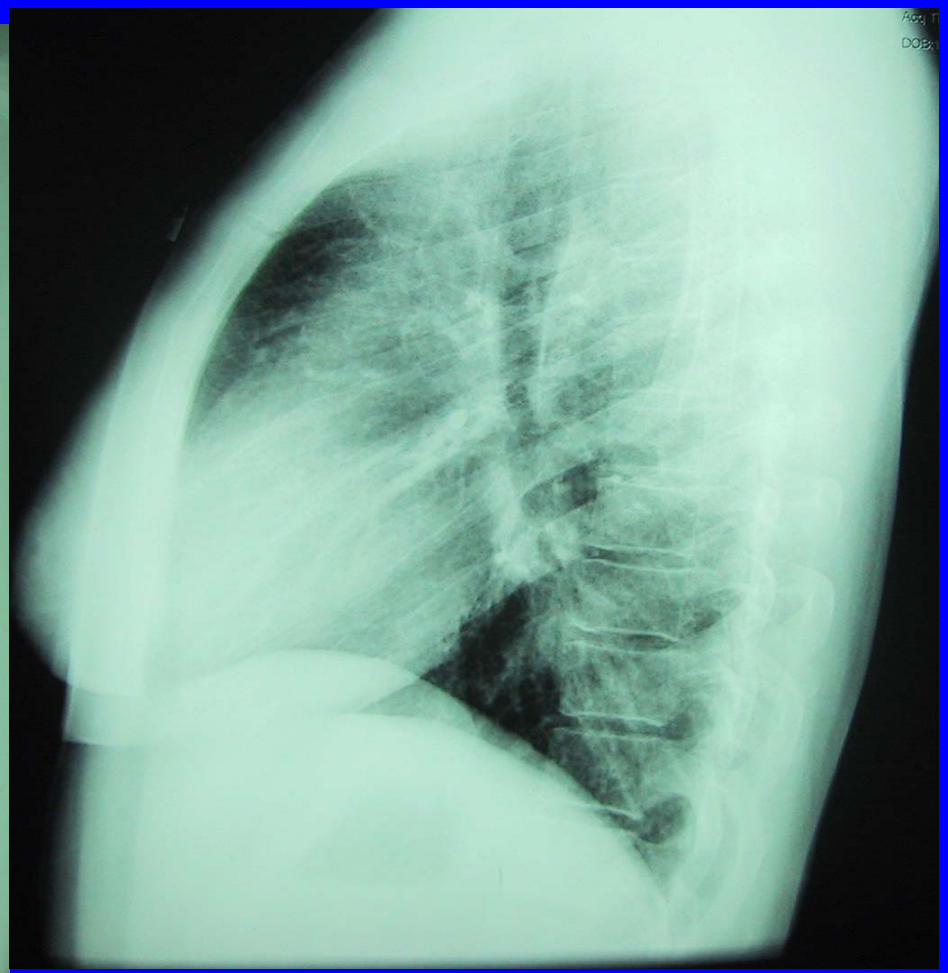
Microbiology (specific defects, specific bugs)

23 yo woman; athletic coach

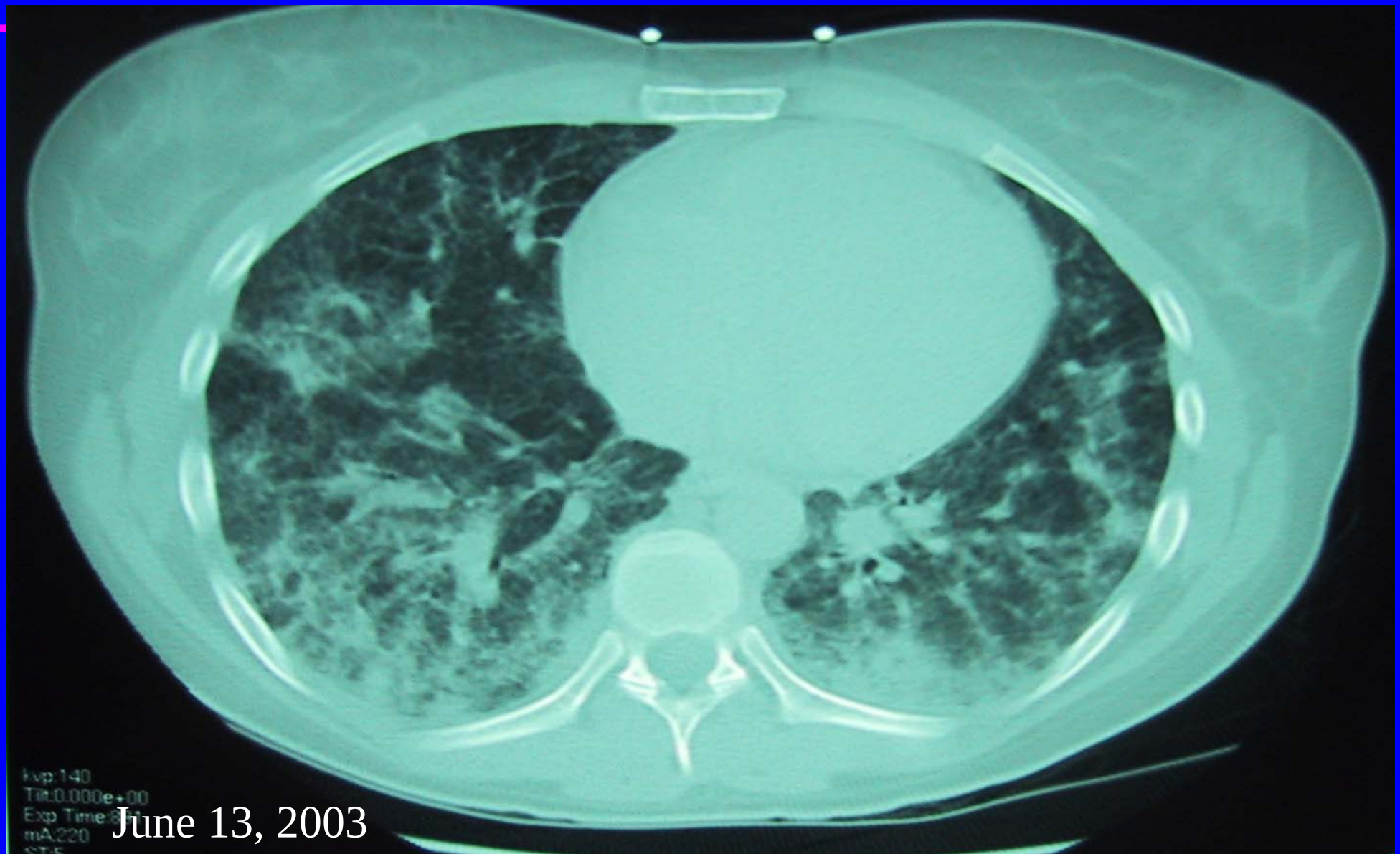
Previously healthy; short of breath 4 hours after 3 mile run



June 11, 2003



Low oxygenation and fever



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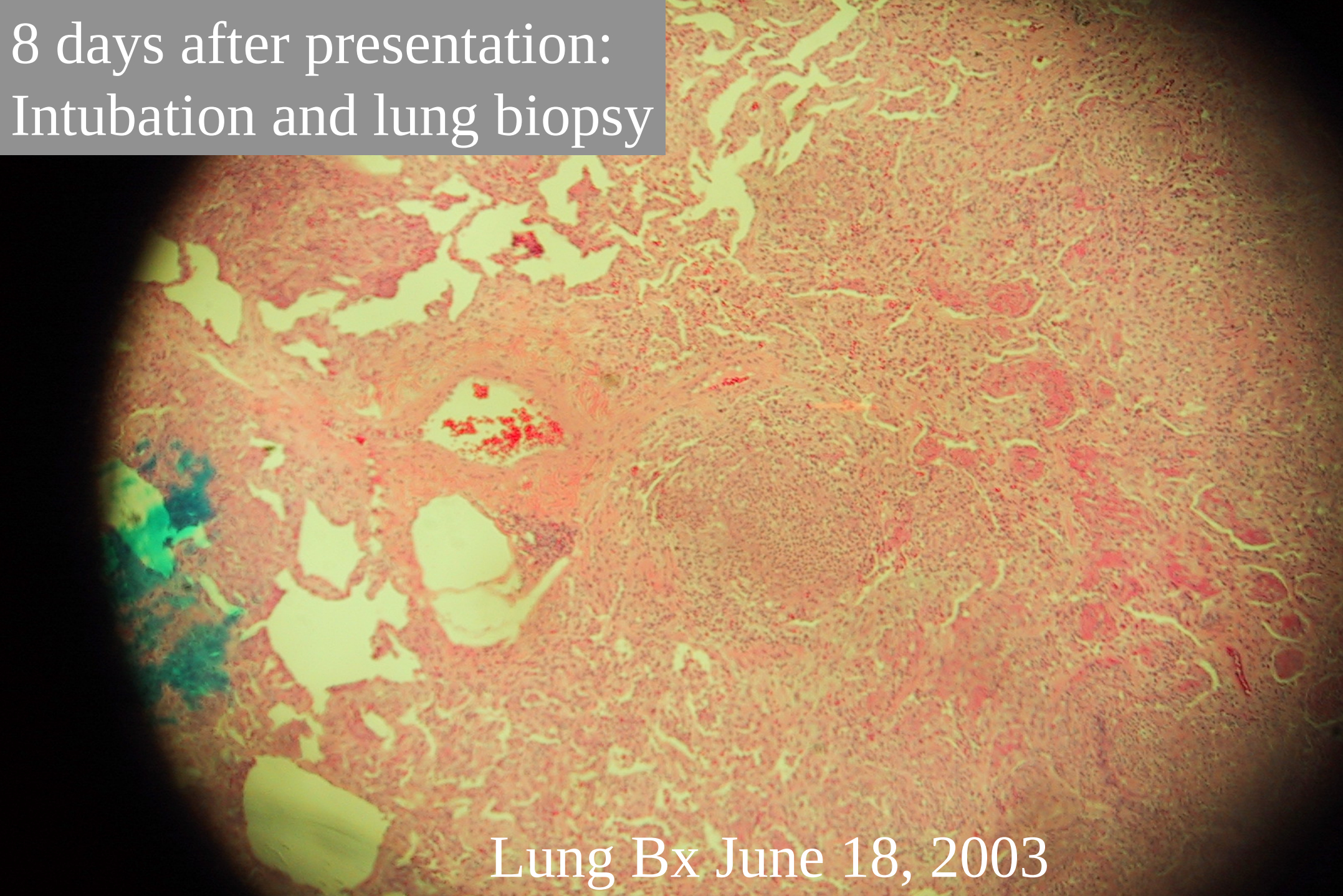
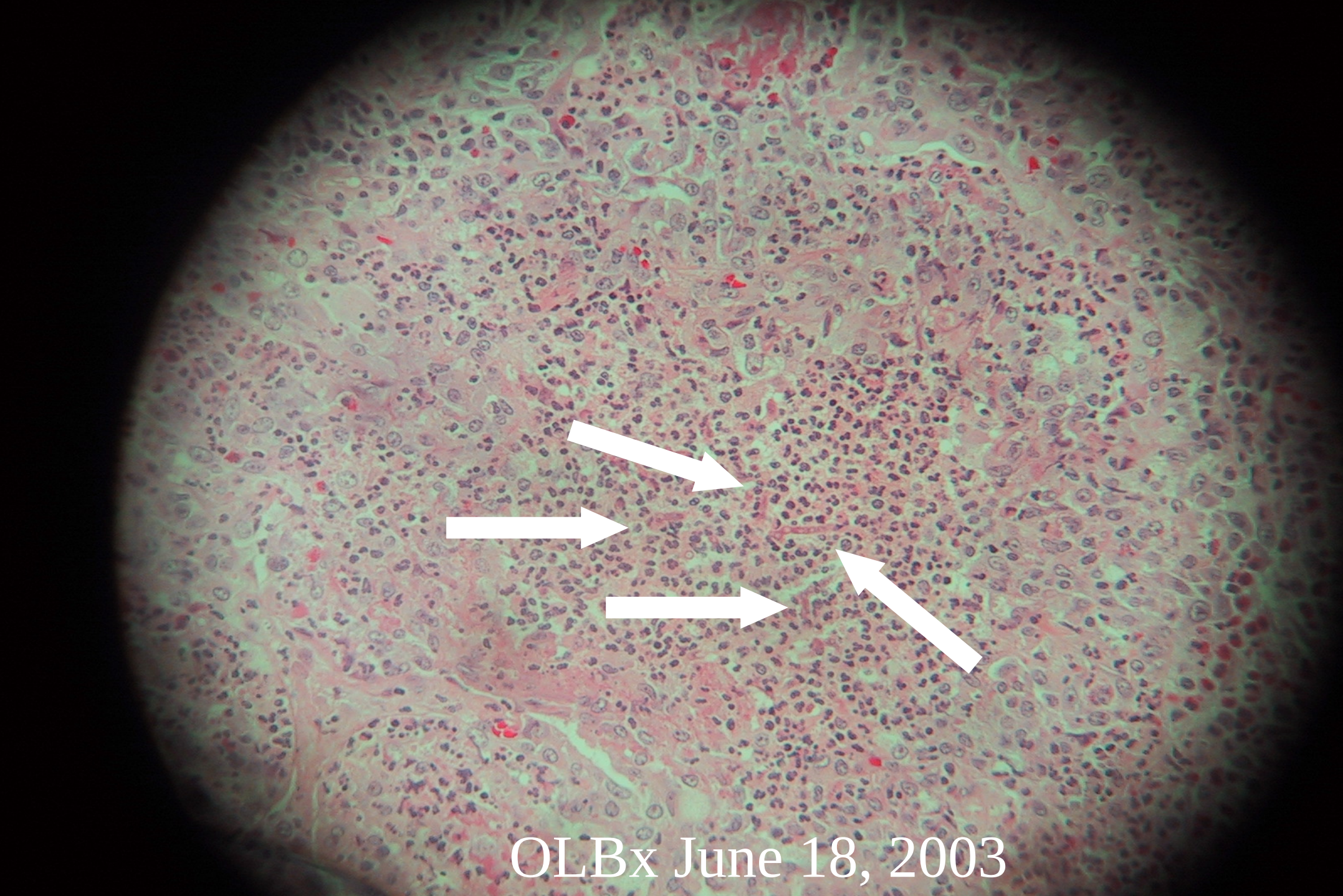

8 days after presentation:
Intubation and lung biopsy
Lung Bx June 18, 2003


OLBx June 18, 2003

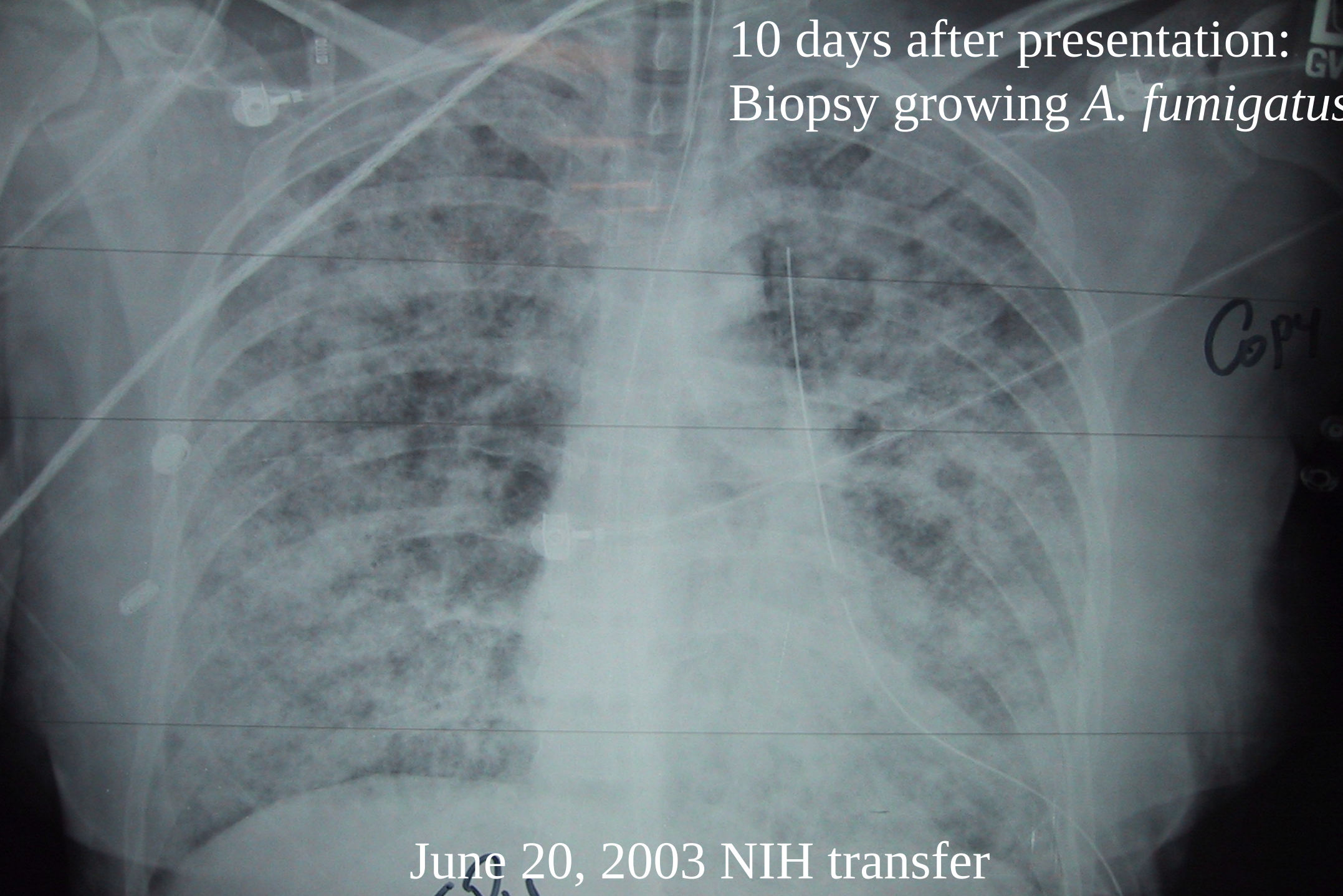
Hospital Course

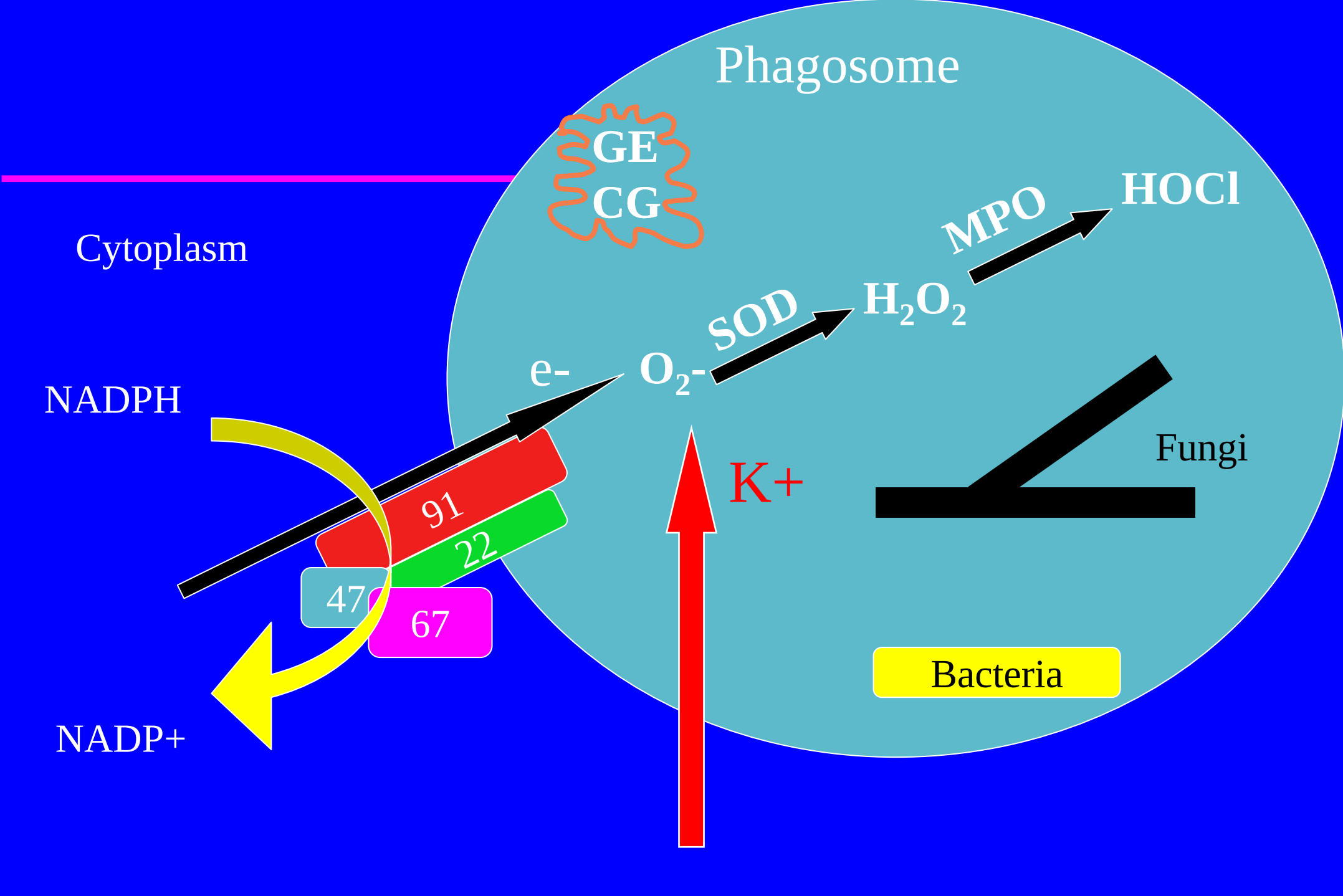
Initiate amphotericin B lipid complex

Progressive hypoxia

steroids, proning, FiO2 80-100%

Aspergillus growing from biopsy


10 days after presentation:
Biopsy growing *A. fumigatus*
June 20, 2003 NIH transfer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CG
Cytoplasm
NADPH
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HOCl
K+
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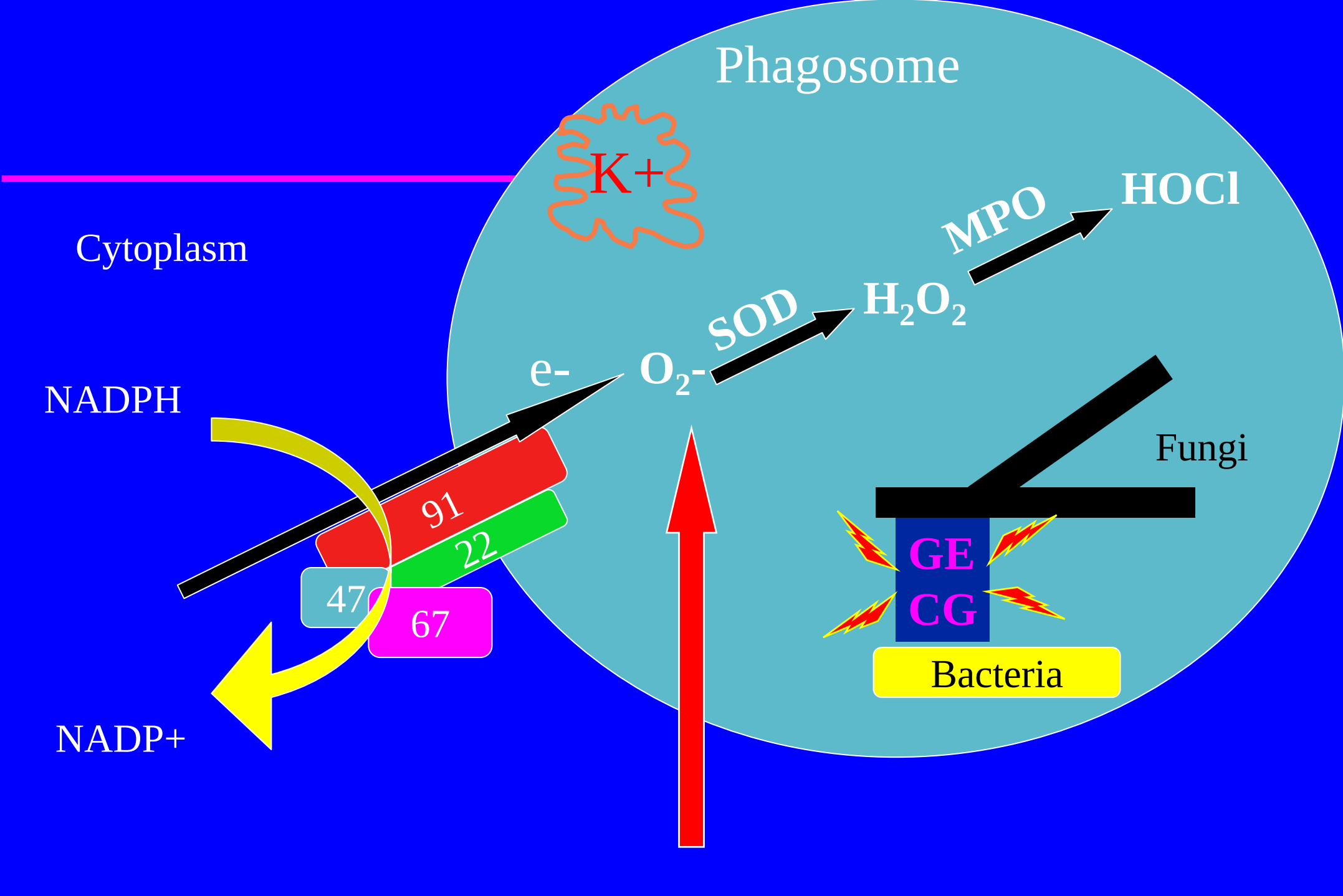
Bacteria
NADP+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K+
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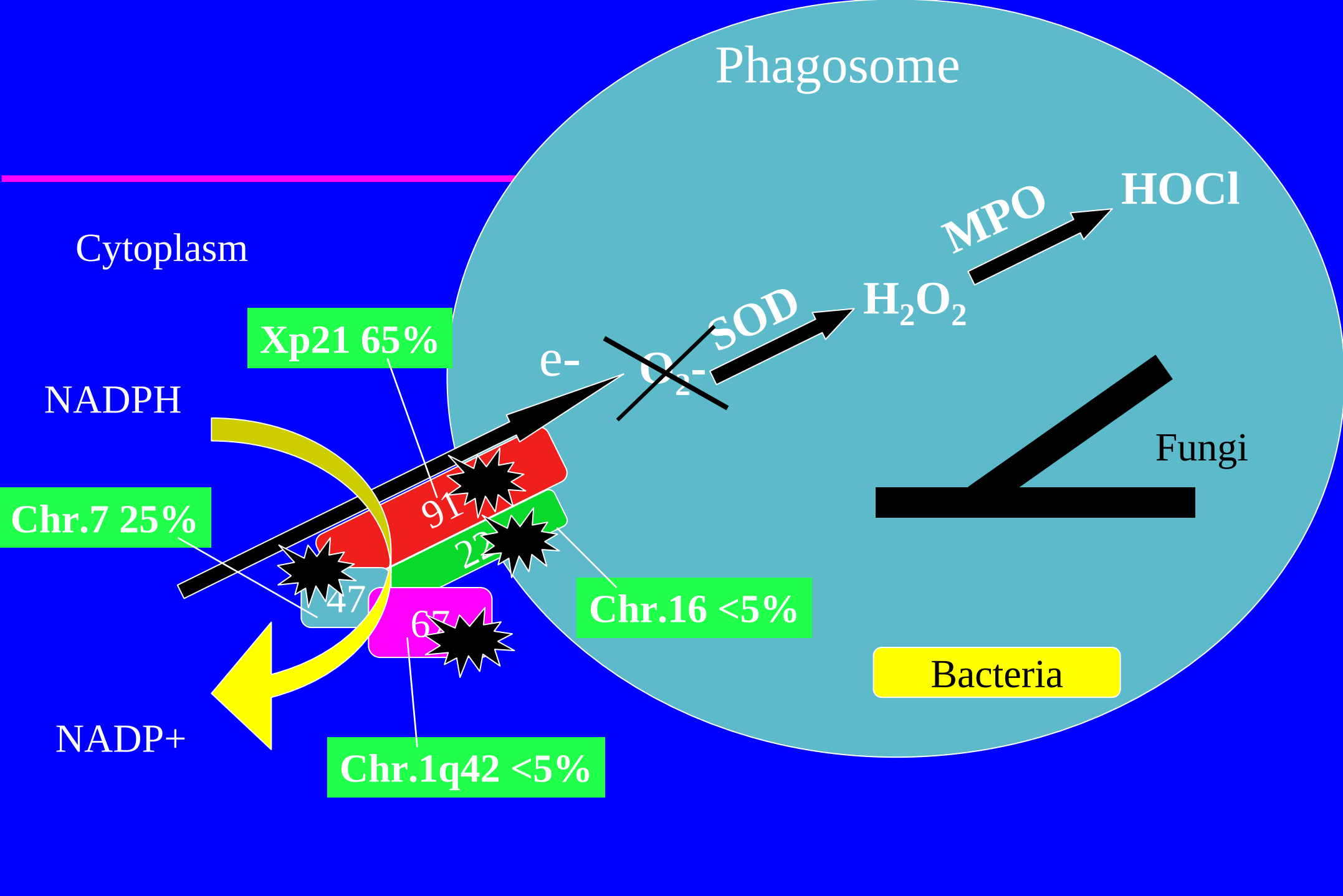
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Xp21 65%
Chr.7 25%
Chr.1q42 <5%
Chr.16 <5%
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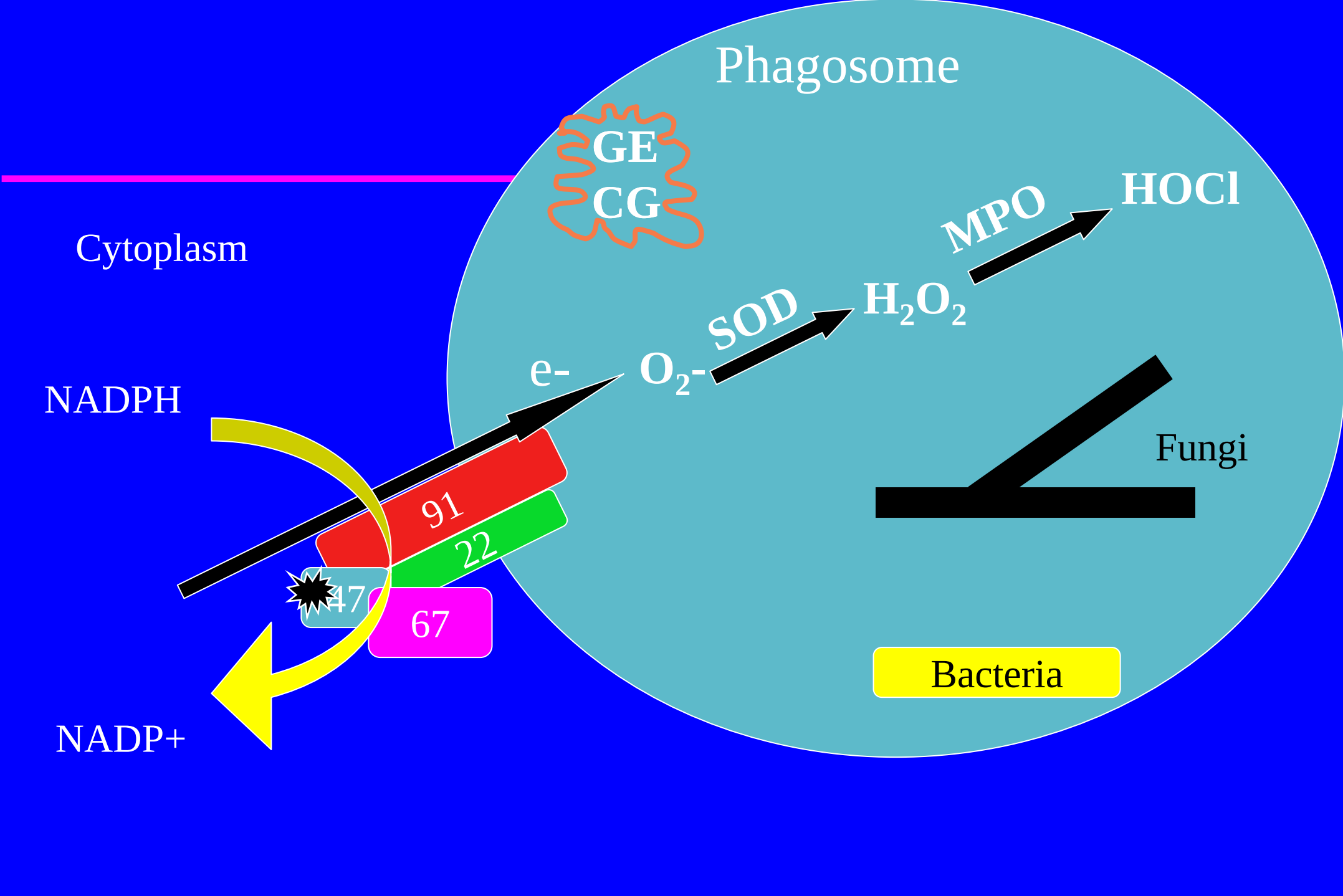
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22
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67
Bacteria
NADP+

Infections in CGD

S. aureus (liver, lymph nodes,osteo)

S. marsescens (skin, lung, lymph nodes)

B. cepacia (pneumonia, bacteremia)

***Nocardia* spp.** (pneumonia, brain, liver)

***Aspergillus* spp.** (lung, esp. miliary, spine)

Rare but pathognomonic infections:

Chromobacterium violaceum *(warm brackish water, soil, e.g., Disney World)*

***Paecilomyces* spp.**

All the above are catalase +; <u>BUT</u> Most bacterial pathogens are catalase +

What makes these different?

Why do CGD patients get granulomatous complications?

Failure to degrade inflammatory mediators, since superoxide and hydrogen peroxide control the degradation of:

C5a

LTB4

fMLF

Failure to properly activate cathepsin G, elastase

Other mechanisms: Low IDO activity, low kynurenine, high IL-17

Treatment: Steroids

Further History

- Mulching on the day of admission for several hours
- Then ran 3 miles
- Had mulched previously

Follow up

Voriconazole, Caspofungin
Steroids, NO

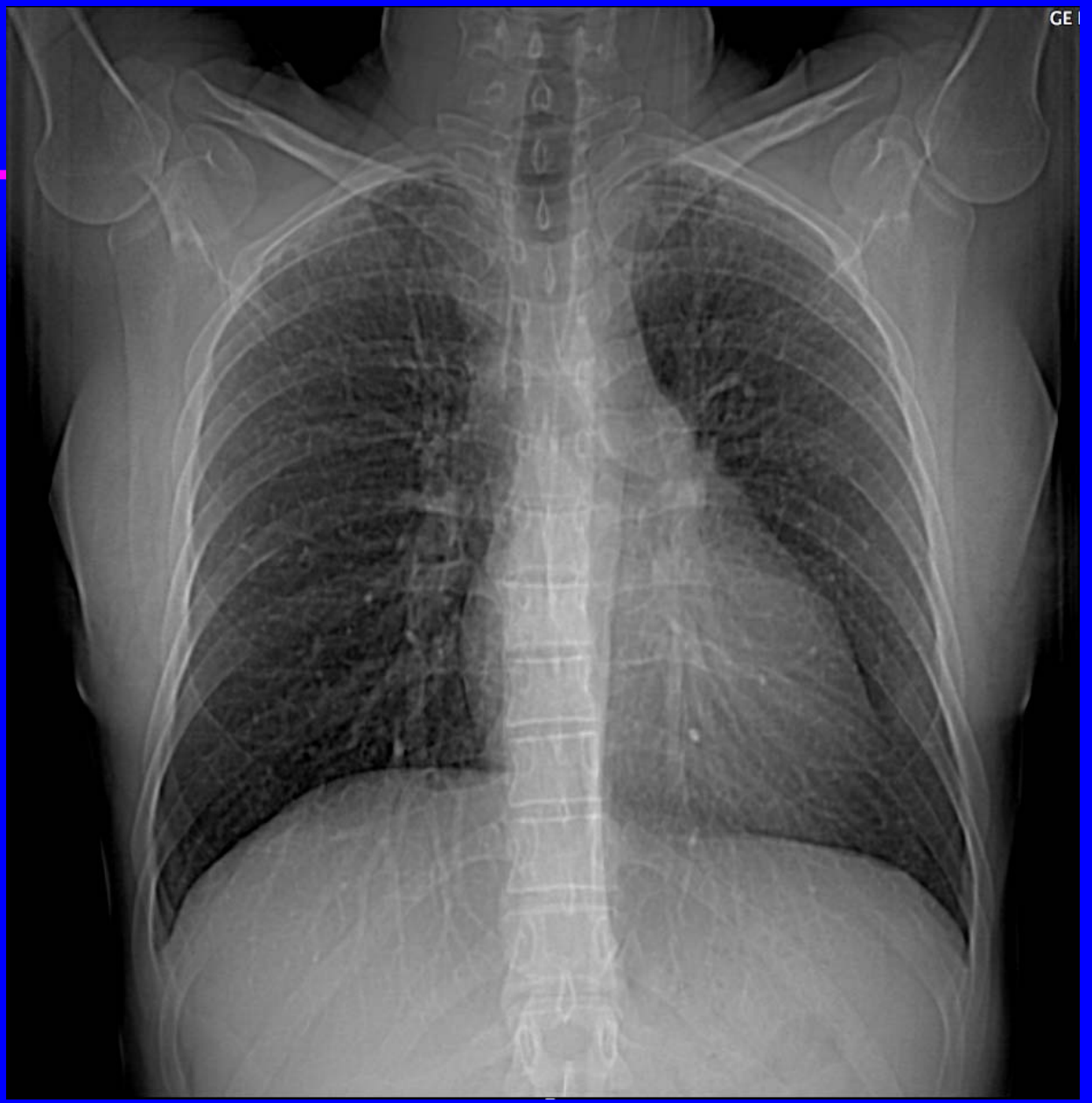
Pneumothoraces,
Inflammation with steroid taper

Complete resolution

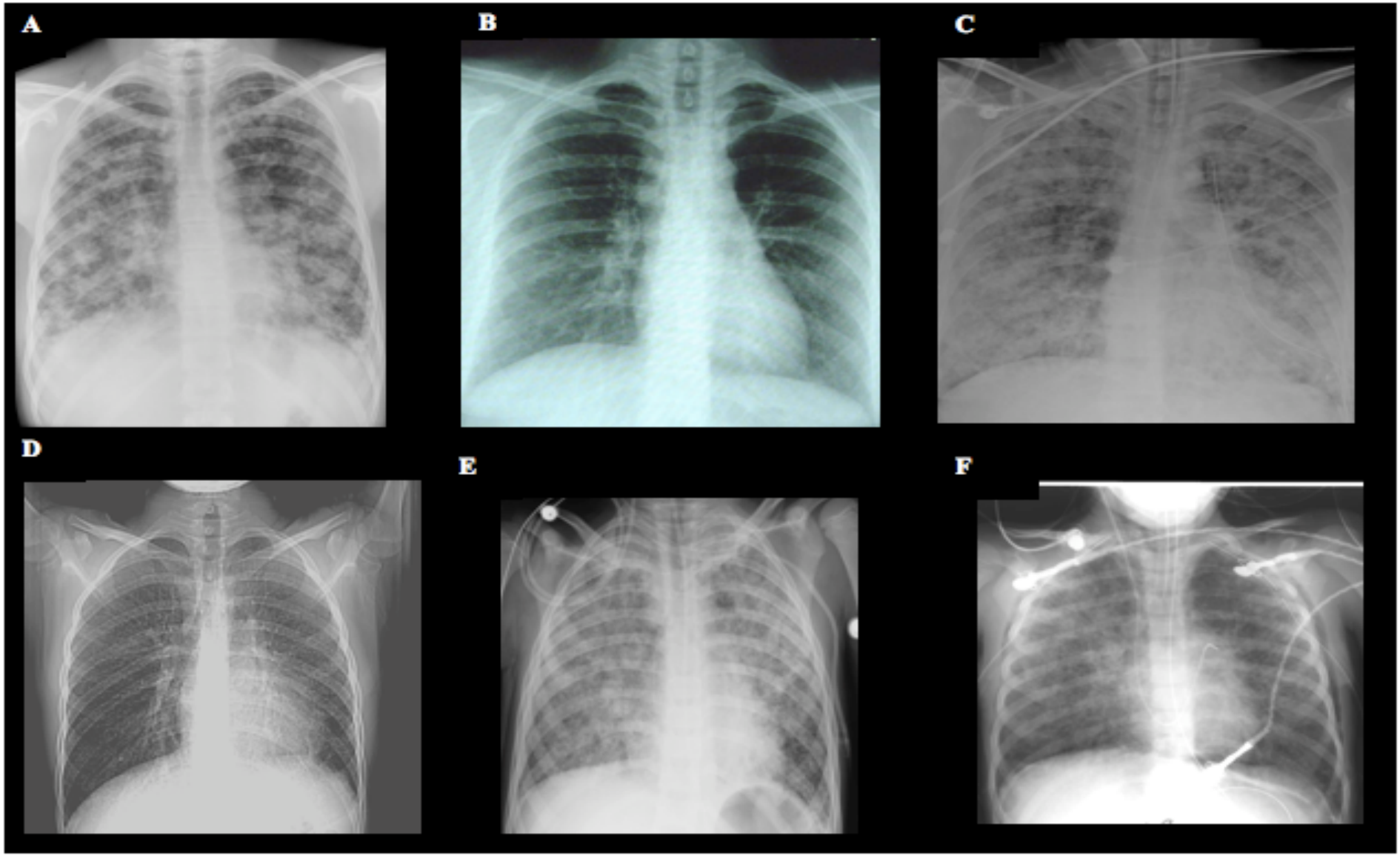
Normal PFTs

Working full time

Running 4 miles/d



Fulminant Mulch Pneumonitis in CGD



Clin Infect Dis 2007 Sep 15;45:673-81

Fulminant CGD Pneumonitis

8 patients 1F/7M

ages 8, 10, 14, 16, 18, 23, 23, 64 yrs

4 p47^{phox} 4 gp91^{phox} deficient

6 autumn 2 summer

Outcome

4 died

3 during initial hospitalization

1 after transfer to nursing home

64 year old man after 1 year hospitalization

4 survived

all returned to normal lung function

hospitalizations around 30 days

Fulminant CGD Pneumonitis

Think about it in older children and adults with acute, diffuse, miliary disease with hypoxia

Use steroids early and continue them longer than usual. Then go longer.

Good antifungals and steroids are the most important

Push for a good history

Inflammation in CGD is NOT dependent on live fungi

What do the steroids regulate?

CGD Take Home messages

Mulch inhalation causes an acute syndrome of hyperinflammatory pneumonitis in CGD

Superoxide is critical for protection against a small number of organisms

Antibiotic and antifungal prophylaxis work

Resistance induced by prophylaxis is rare in CGD

Why is this experience different than other diseases?

JOB'S SYNDROME

Recurrent, "Cold", Staphylococcal Abscesses

STARKEY D. DAVIS
M.D. Baylor
ASSISTANT PROFESSOR

JANE SCHALLER
M.D. Harvard
INSTRUCTOR

RALPH J. WEDGWOOD
M.D. Harvard
PROFESSOR AND CHAIRMAN
DEPARTMENT OF PEDIATRICS,
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

"So went Satan forth from the presence of the Lord, and smote Job with sore boils from the sole of his foot unto his crown".—*Job*, II, 7.

WE have examined two girls who have had recurrent, "cold", staphylococcal abscesses since birth. The staphylococci do not seem to be unusually virulent, and neither child has diabetes or any other condition known to predispose to infection. Since we are not aware that any similar cases have been described previously, we report these cases in detail.

EXTREME HYPERIMMUNOGLOBULINEMIA E AND UNDUE SUSCEPTIBILITY TO INFECTION

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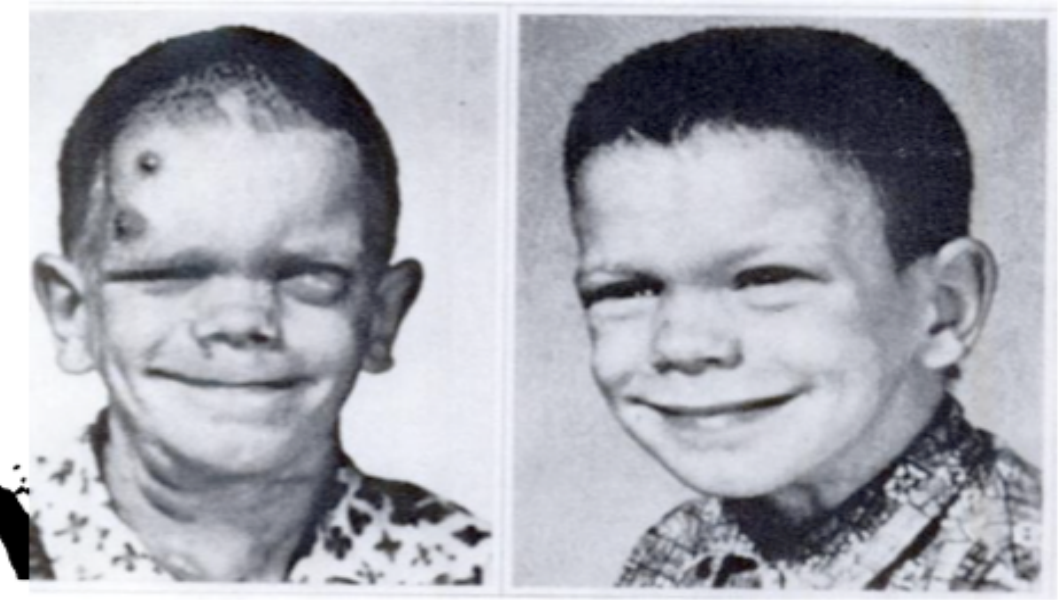
From the Departments of Pediatrics and Microbiology and Immunology, the Duke University School of Medicine, Durham, North Carolina, and the Department of Pediatrics, the Medical College of Georgia, Augusta, Georgia

HYPERIMMUNOGLOBULINEMIA E



. 1. Patient B.S. at 8 years of age, before and after initiation of oxacillin therapy. (Reproduced by permission of Bristol Laboratories).

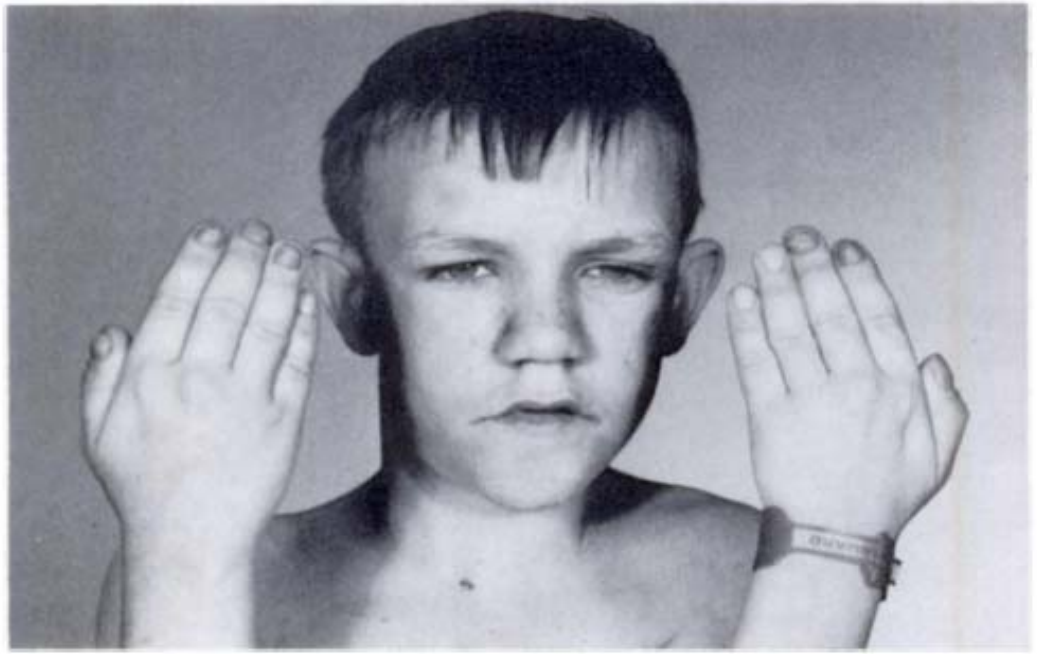


FIG. 3. Patient R.B. at 12 years of age. Mycotic infections of the nails are apparent. Ptosis of the left lid developed following cardiac arrest during lung surgery.

Pediatrics 1972;49;59-70

HIES Aspects

Autosomal dominant and sporadic

Frequency probably $\leq$ 1:100,000

Female = male

Diagnosis usually in childhood

Symptoms begin in infancy

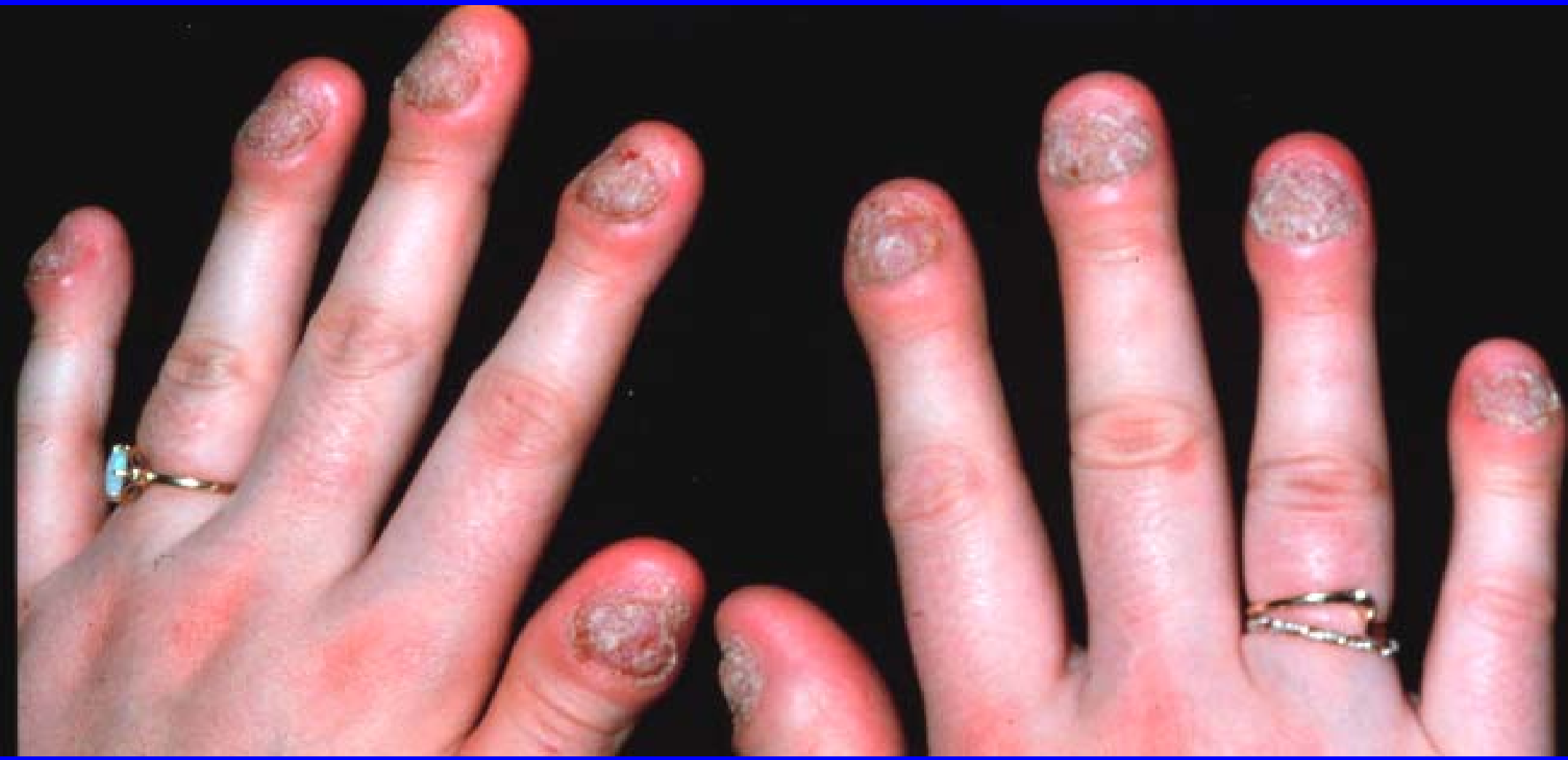
Developmental phenotype

(teeth, facies, scoliosis)

Features of HIES

Eczema	100%
Characteristic facies (>16y)	100%
Skin boils	87%
Pneumonias	87%
Lung cysts	77%
Mucocutaneous candidiasis	83%
Scoliosis (>16y)	76%
Delayed dental deciduation	72%
Brain T2 hyperintensities	70%
Pathologic fractures	57%
Chiari I malformation	18%

Candida albicans onychomycosis



Pulmonary Pathogens in HIE

Primary pathogens:

Staphylococcus aureus

Hemophilus influenzae

Streptococcus pneumoniae

Secondary pathogens:

Pseudomonas aeruginosa

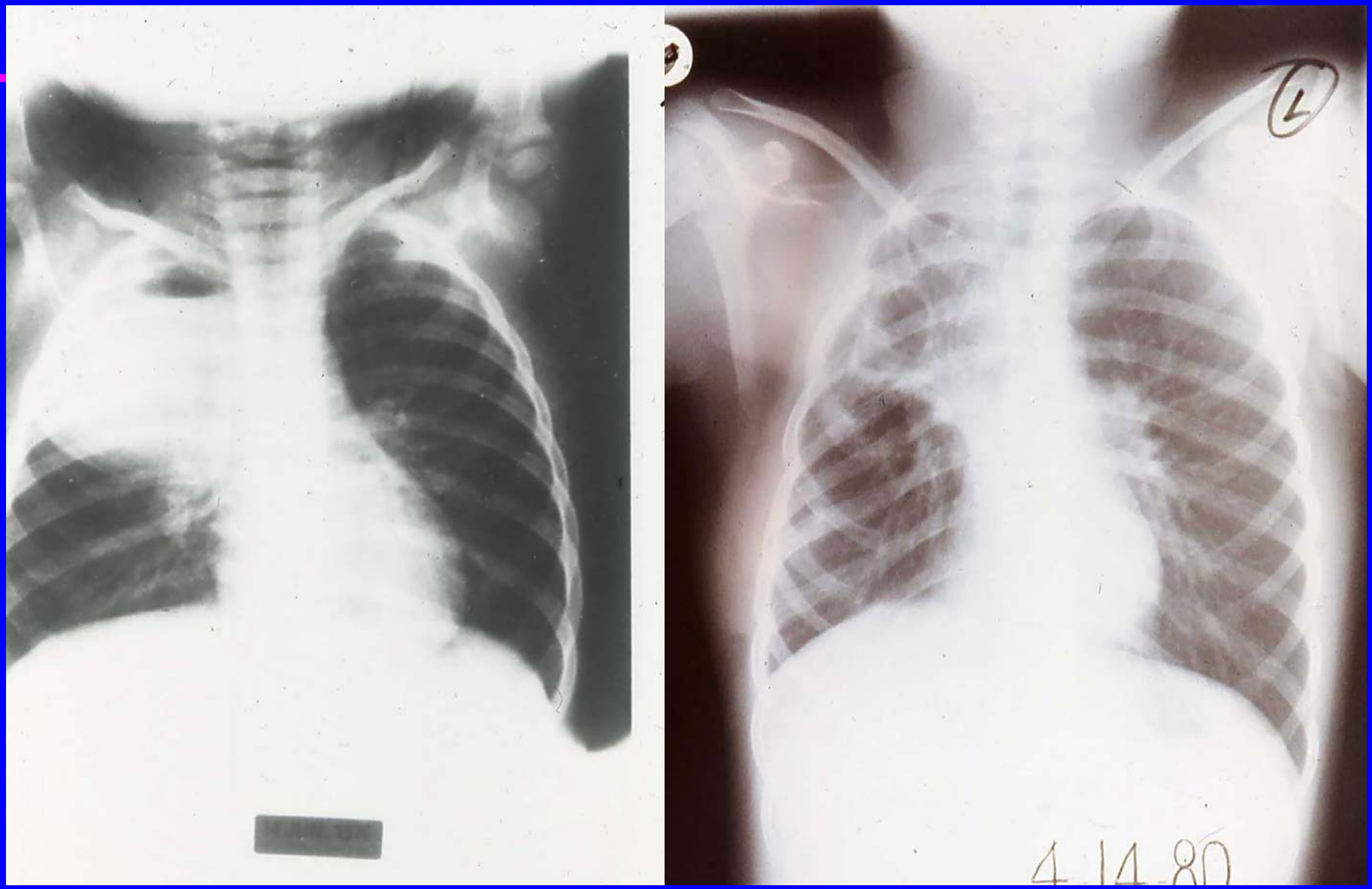
Aspergillus fumigatus

Paecilomyces spp.

Scedosporium spp.

Others:

Pneumocystis jiroveci, *M. avium* complex, *M. kansasii*, *M. abscessus*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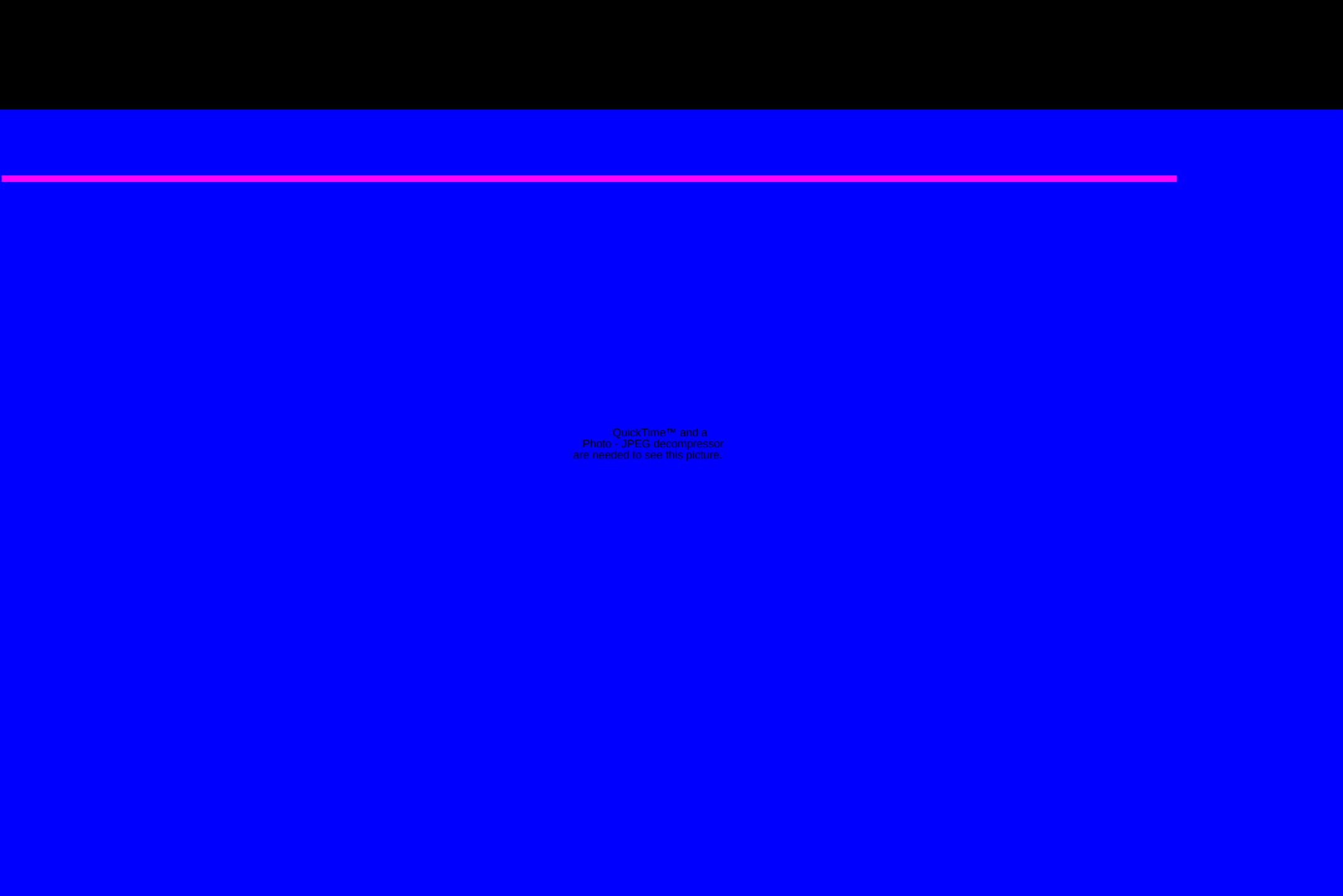
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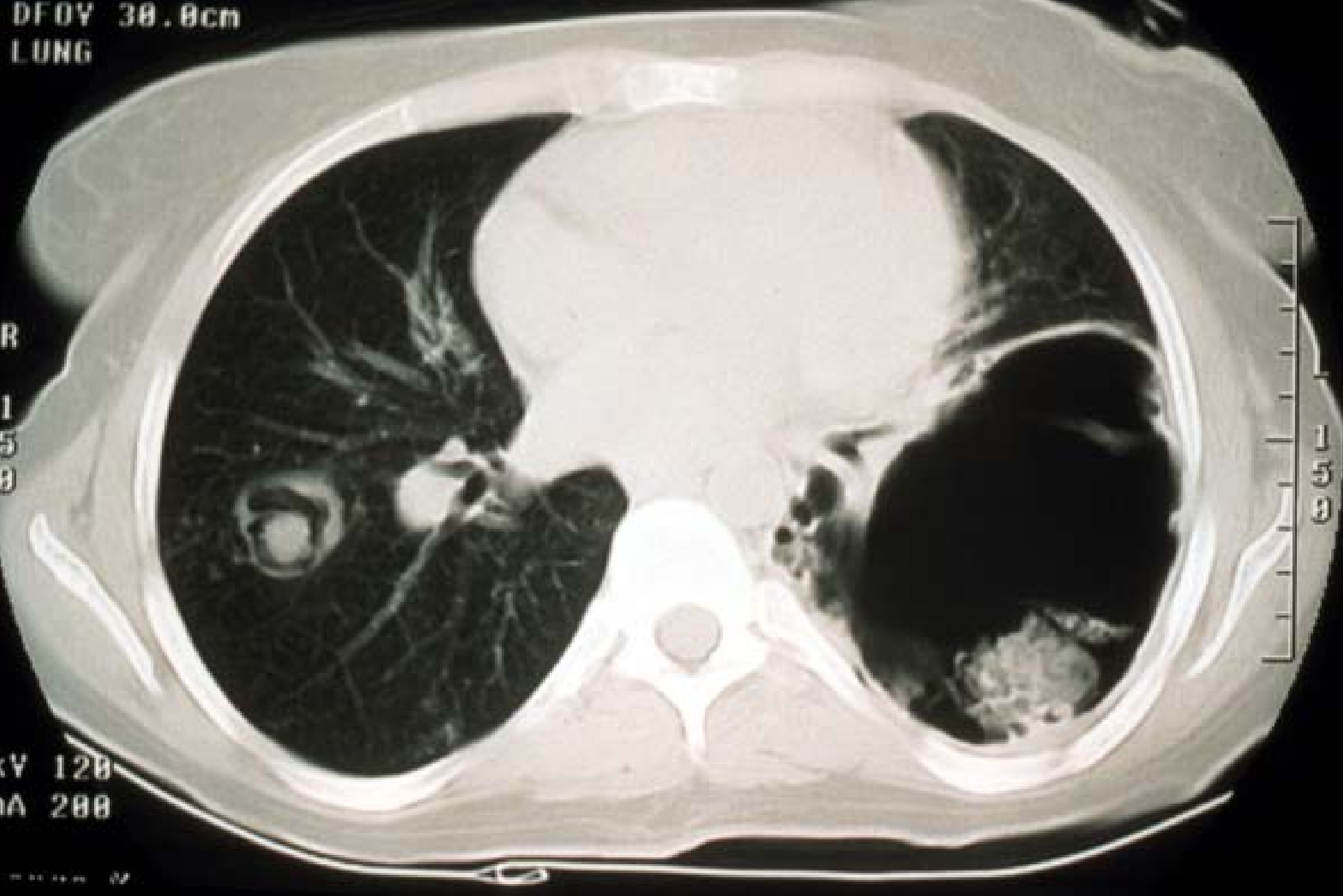
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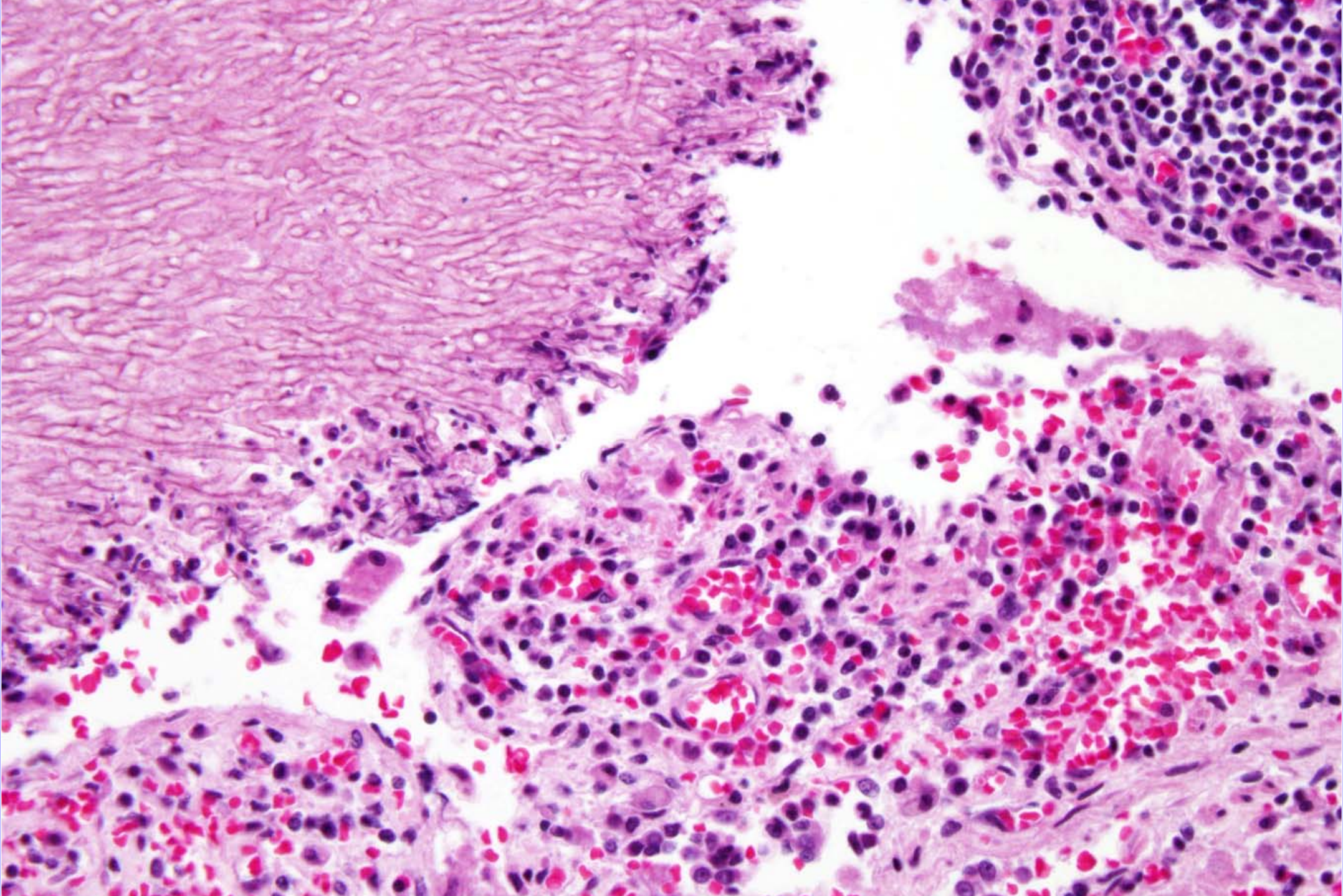
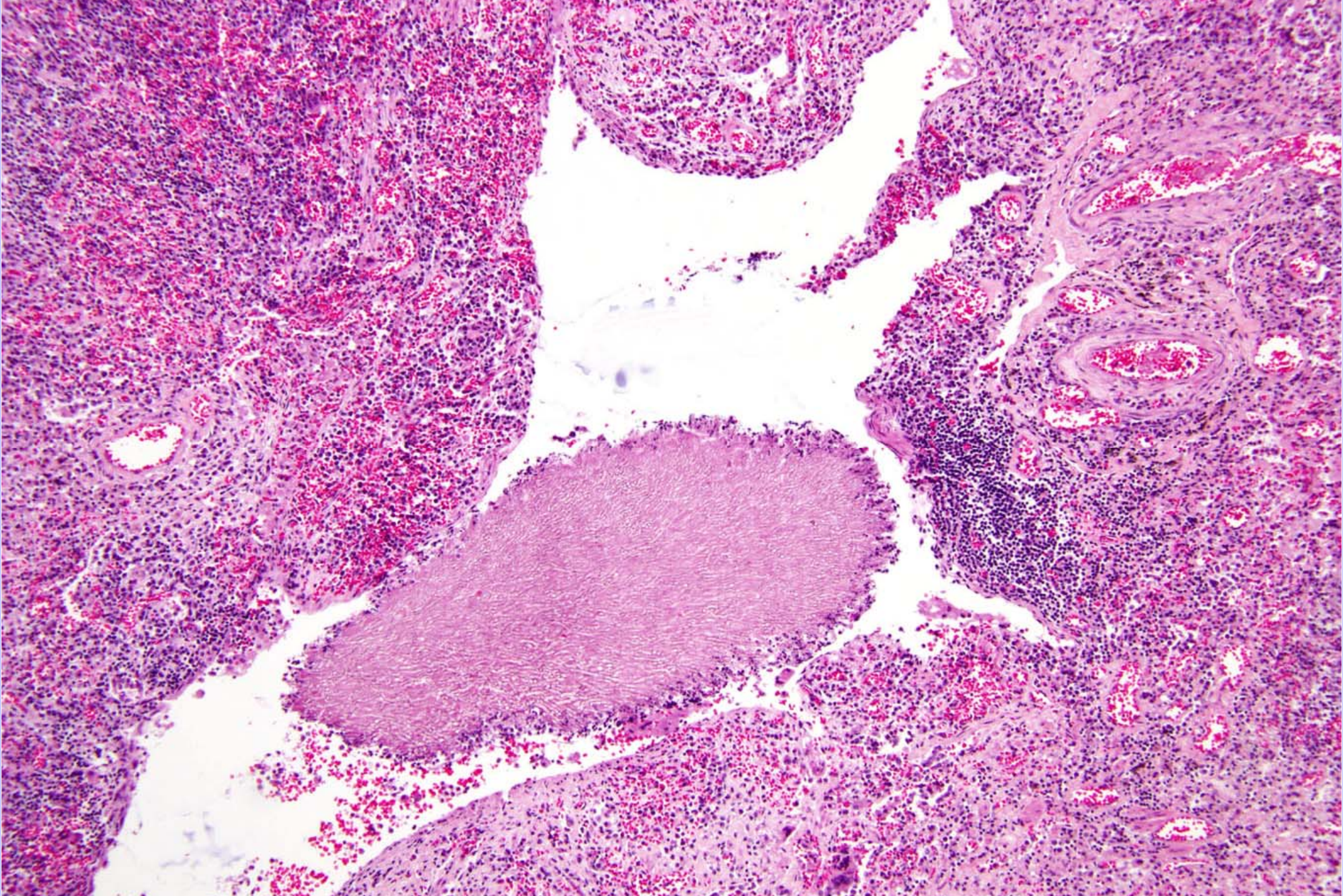
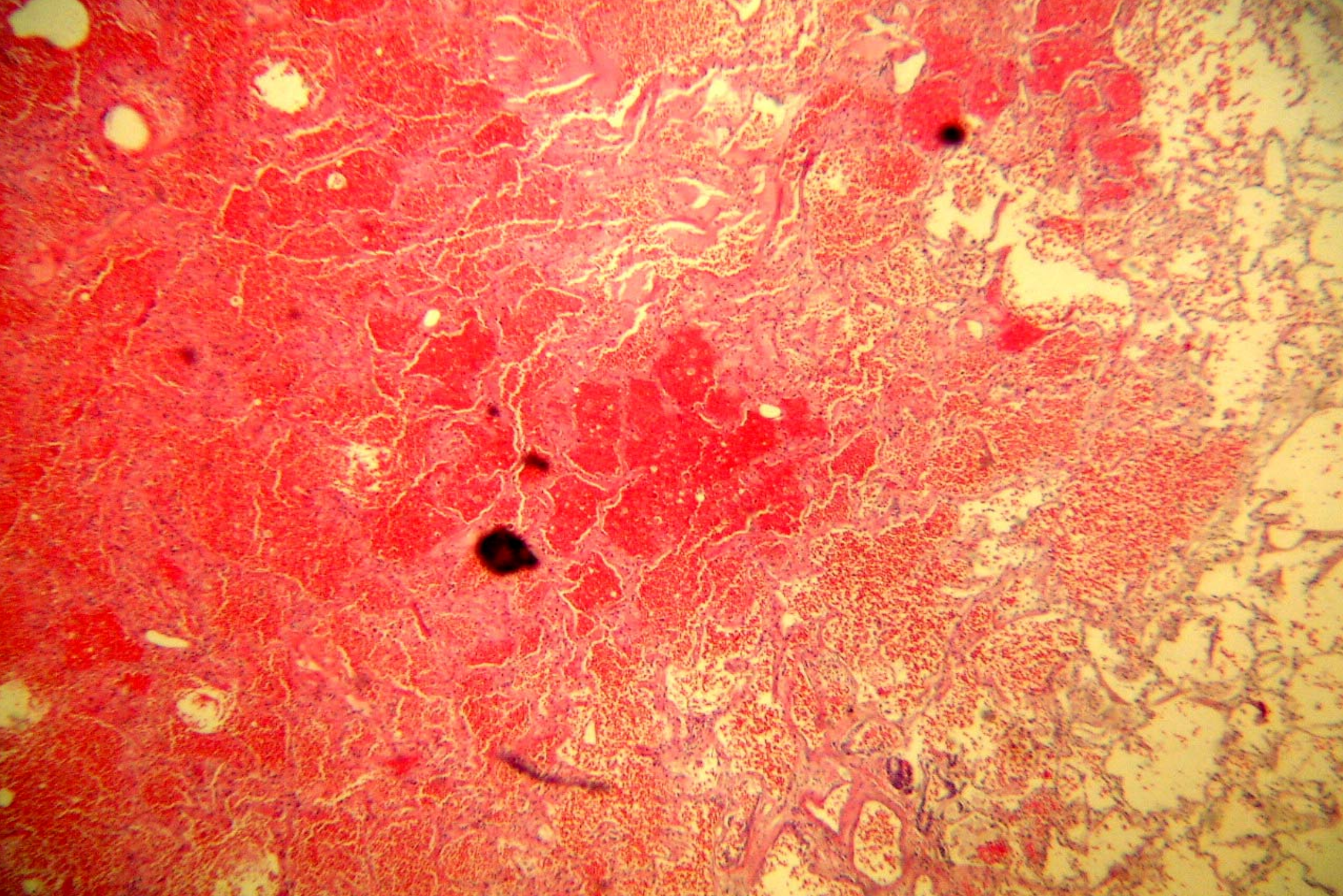
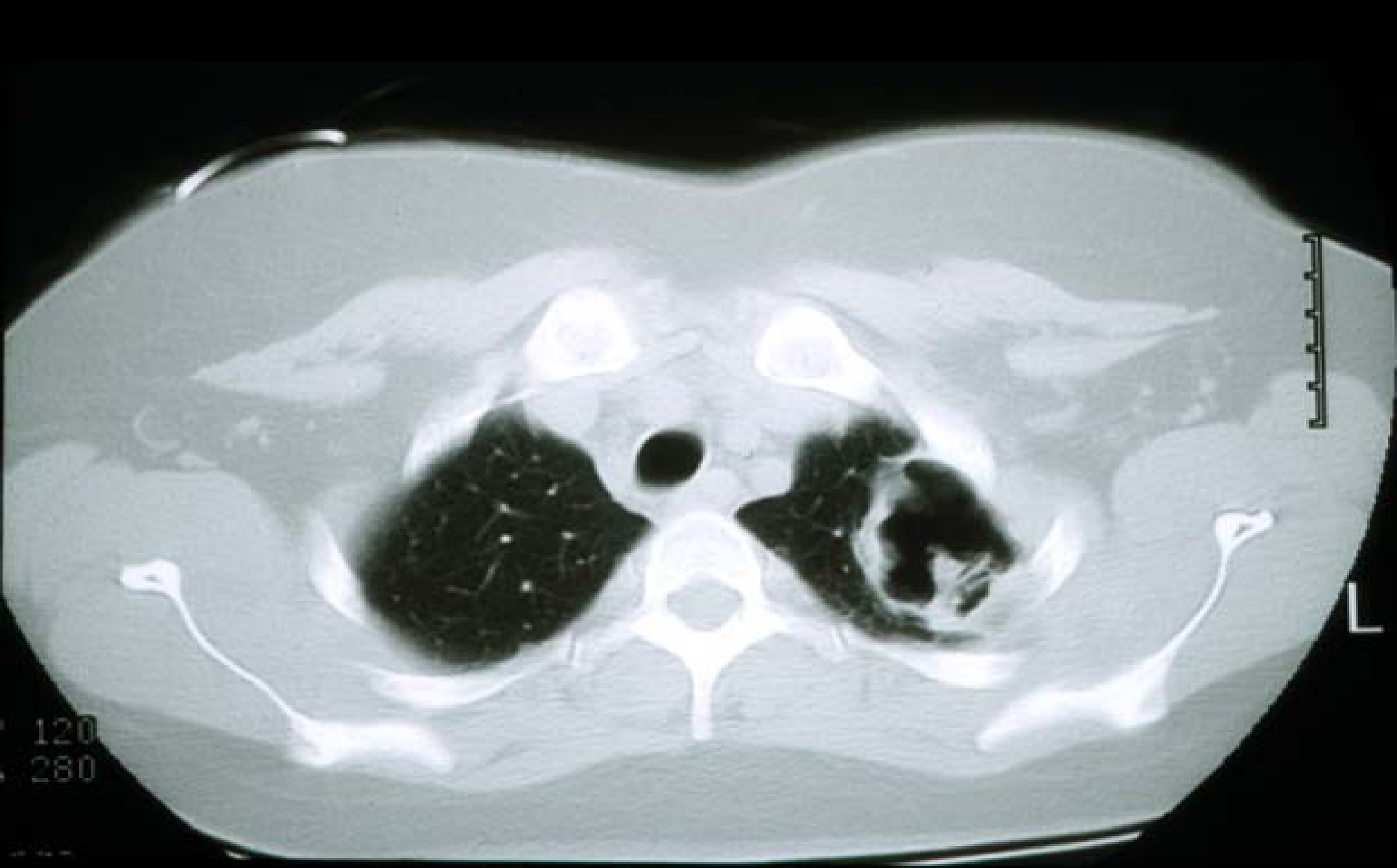
kV 120
mA 200





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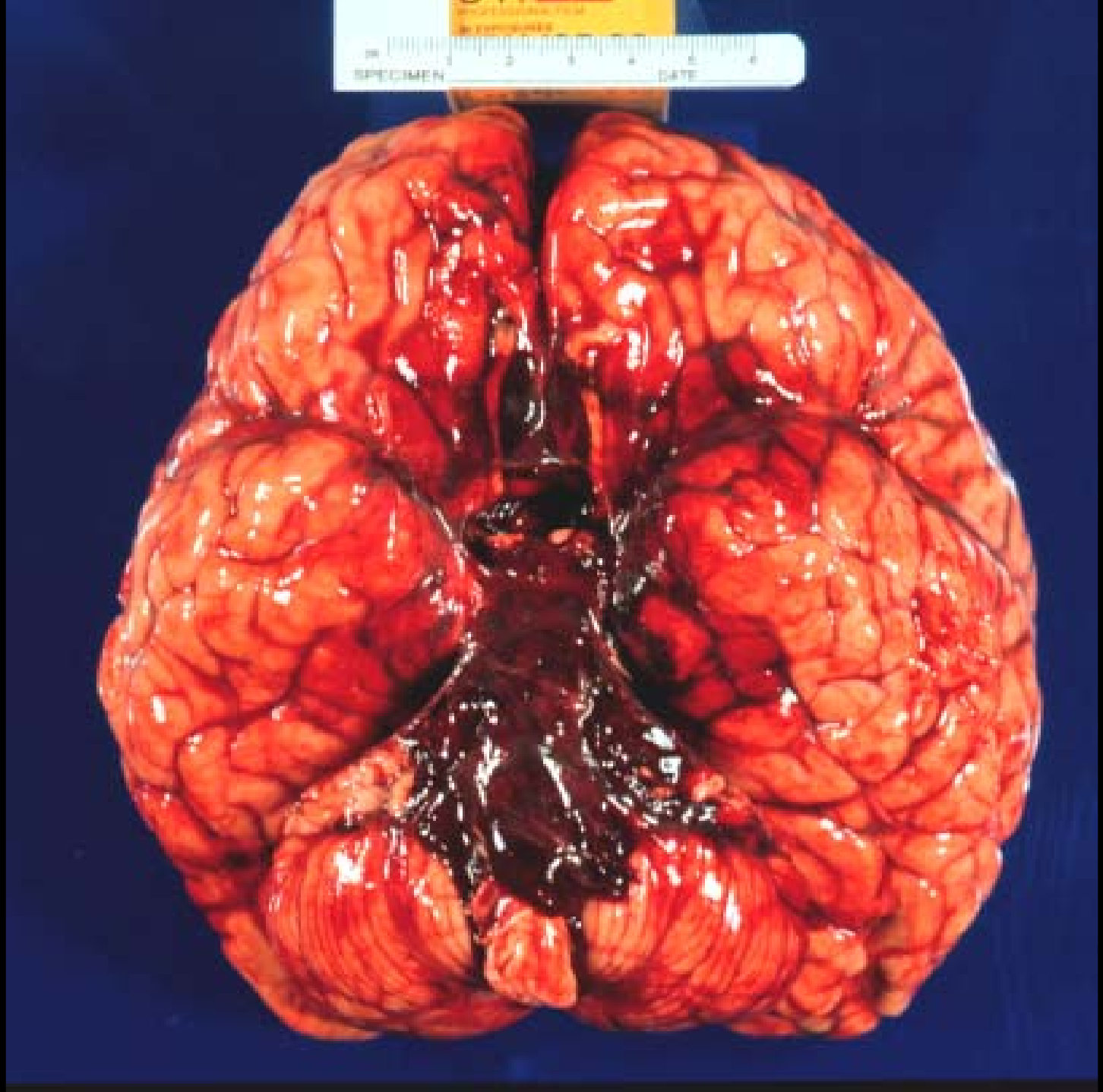
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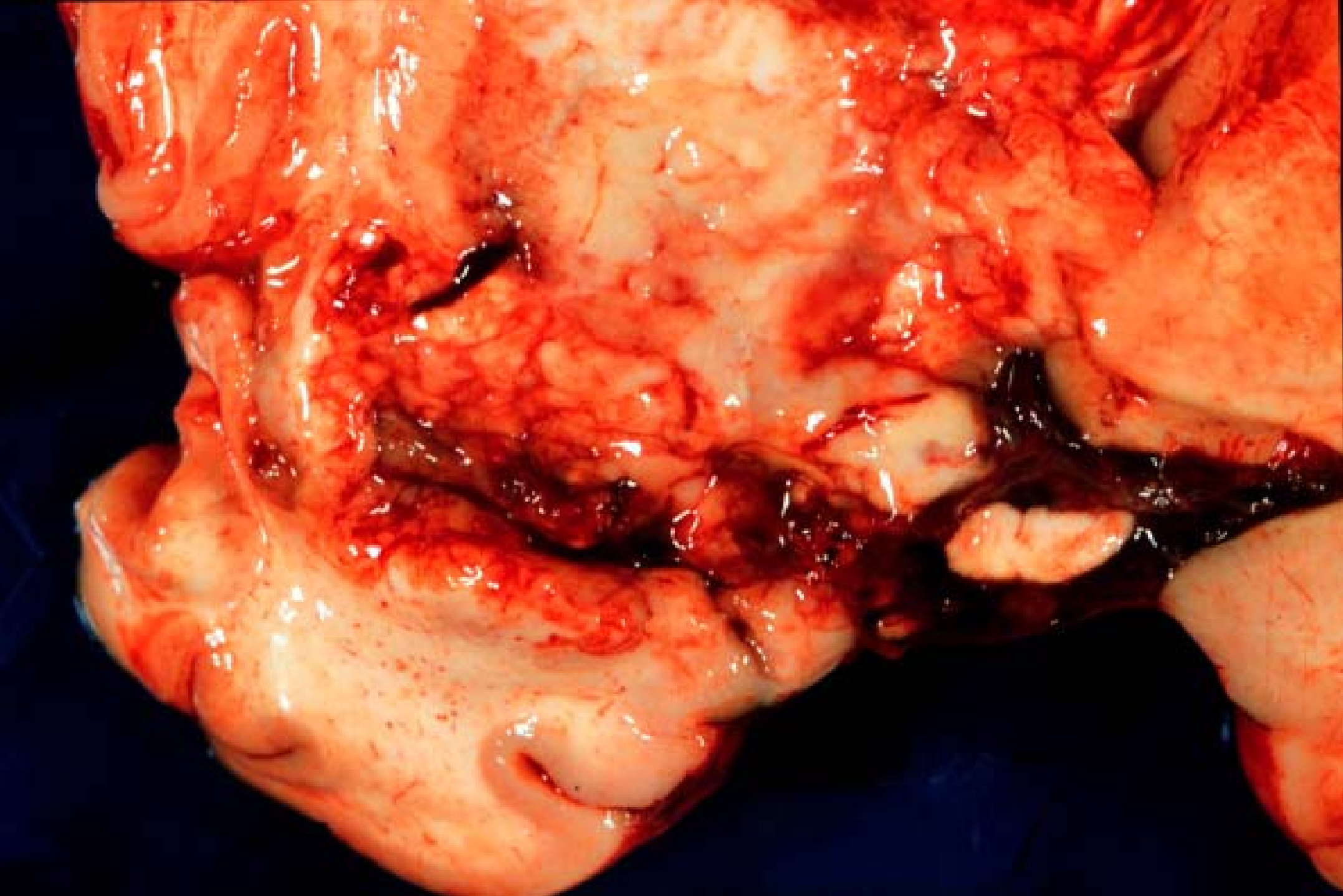
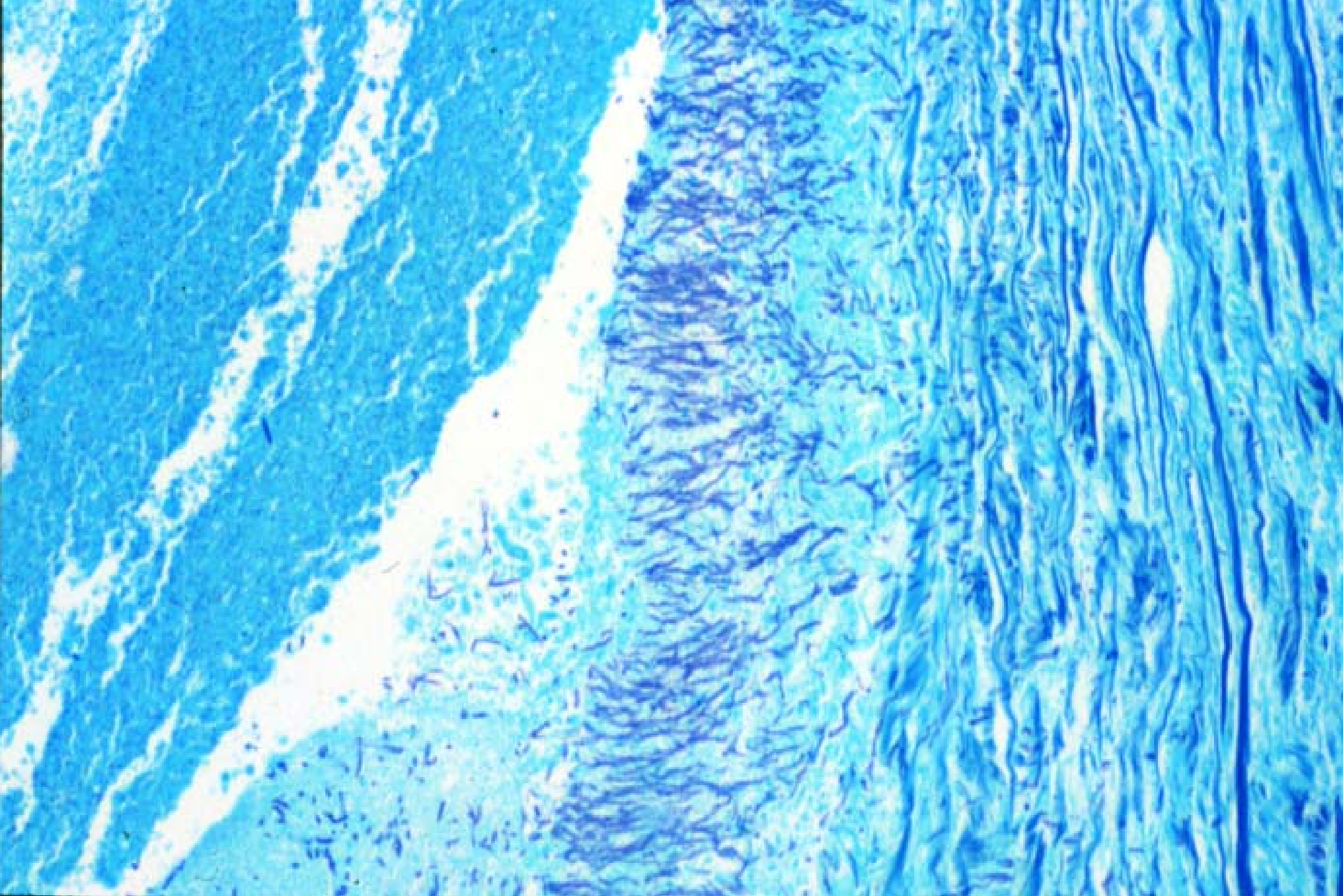




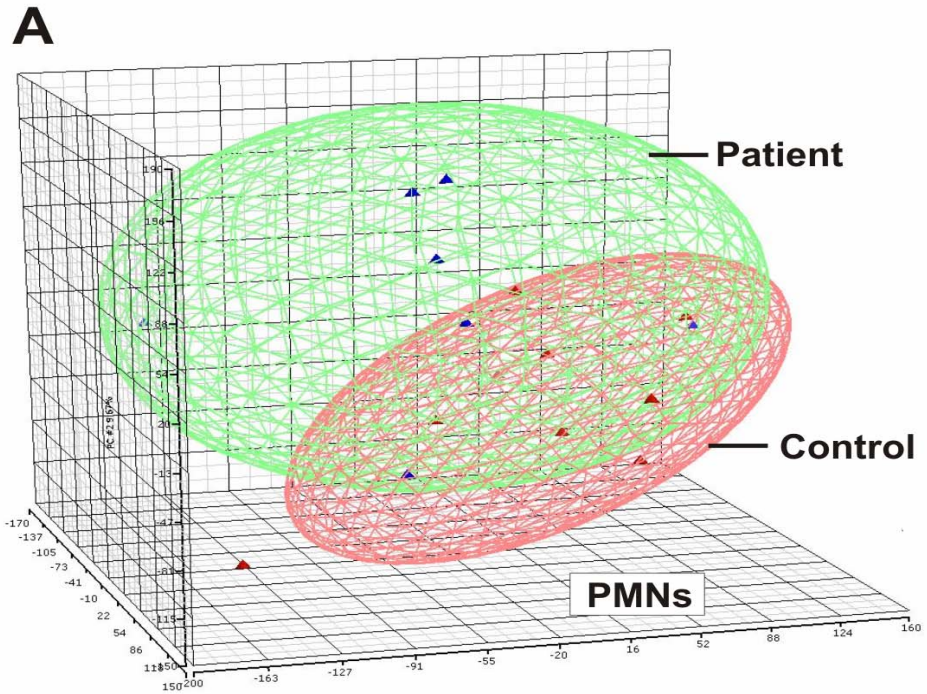
Patient no.	Age at death (y)	Antimicrobials at time of death	Maximum HIES score	Lung cyst	Mucocutaneous candidiasis	Age at first pneumonia (y)	Age at first known fungal pneumonia	Age at first known *Pseudomonas* infection (y)	Lung resection
1	29	Trimethoprim/ sulfamethoxasole, fluconazole	82	Yes	Yes	3	NA	23	Left lower lobe at 4 y followed by left pneumectomy at 15 y
2	24	Vancomycin, meropenem, amphotericin*	71	Yes	Yes	7	*A fumigatus* and *Aspergillus niger* at 23 y	23	No
3	40	Levofloxacin, itraconazole, cefixime	87	Yes	Yes	12	*A fumigatus* at 37 y	36	No
4	24	None†	87	Yes	Yes	2	*A fumigatus* at 18 y	18	Right lower lobectomy at 23 y
5	29	Vancomycin, cefepime, amphotericin	87	Yes	Yes	<10	*A fumigatus* at 27 y	27	Right upper lobectomy at 28 y
6	32	Levofloxacin, itraconazole	68	Yes	Yes	18	*A fumigatus* at 31 y	NA	Left lower lobectomy at 31 y

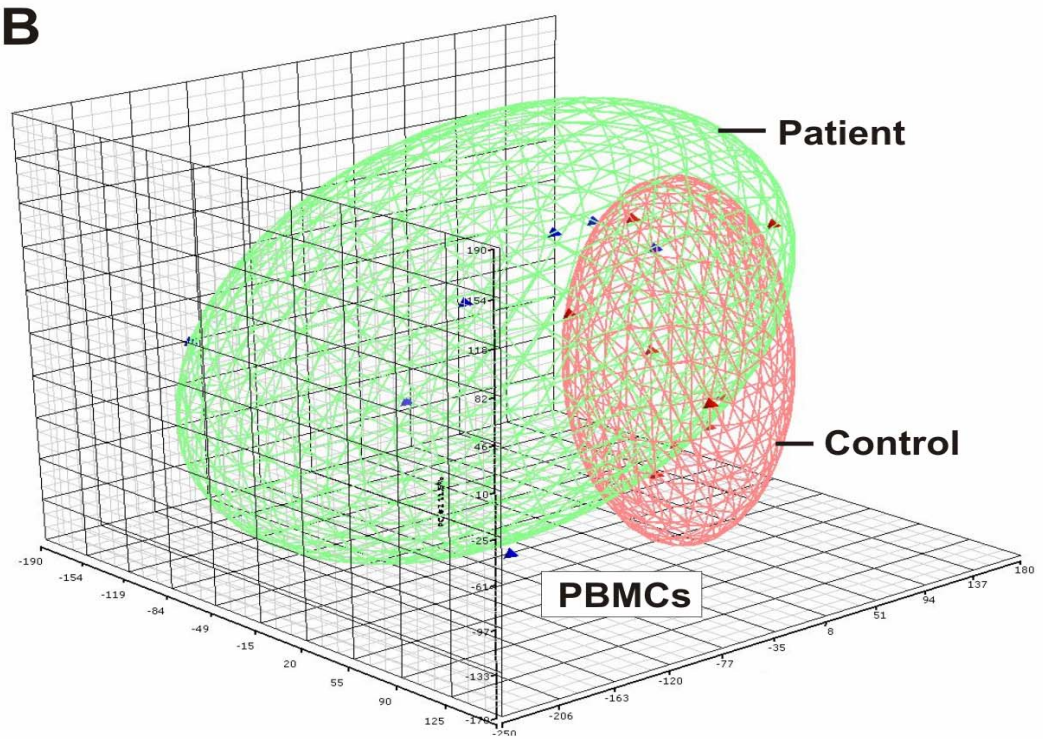
Freeman AF, et al. *JACI* 2007

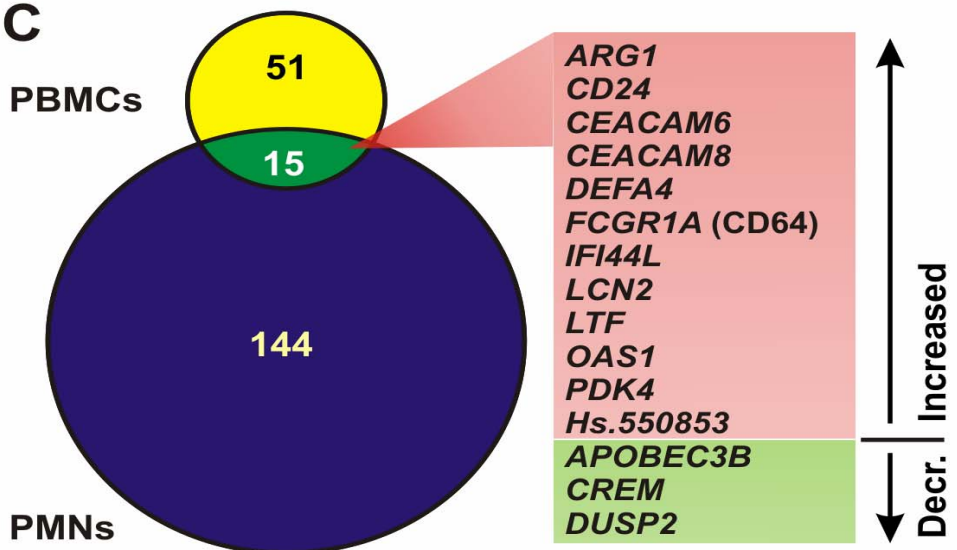
What Single Gene Causes All This?

Eczema	100%
Characteristic facies (>16y)	100%
Skin boils	87%
Pneumonias	87%
Lung cysts	77%
Mucocutaneous candidiasis	83%
Scoliosis (>16y)	76%
Delayed dental deciduation	72%
Brain T2 hyperintensities	70%
Pathologic fractures	57%
Chiari I malformation	18%

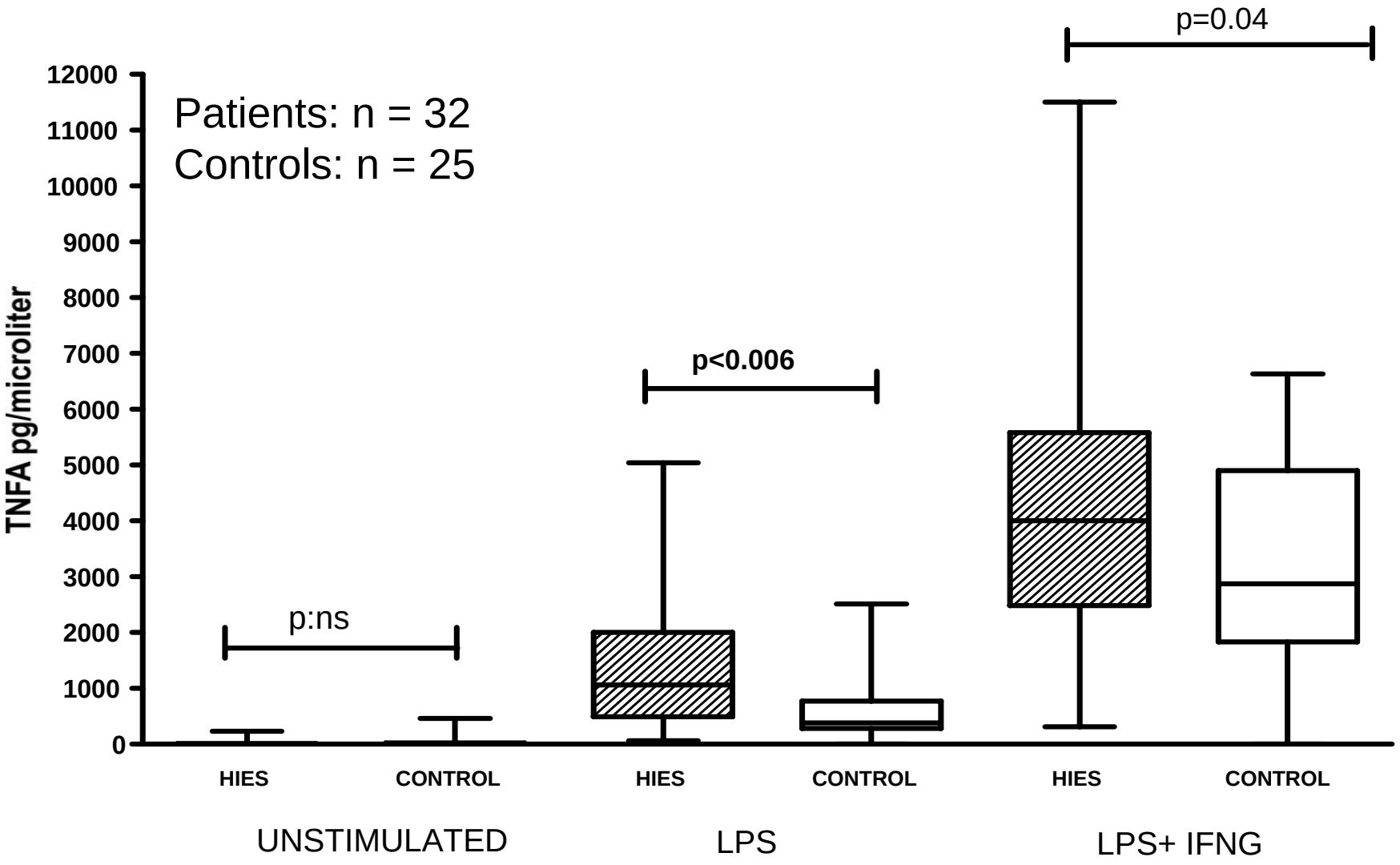
Unstimulated Leukocytes







TNFα Production is Elevated in HIES: LPS



Immunity 25, 745–755, November 2006 ©2006 Elsevier Inc. DOI 10.1016/j.immuni.2006.09.009

Human Tyrosine Kinase 2 Deficiency Reveals Its Requisite Roles in Multiple Cytokine Signals Involved in Innate and Acquired Immunity

Yoshiyuki Minegishi,[1,*] Masako Saito,[1] Tomohiro Morio,[2] Ken Watanabe,[3] Kazunaga Agematsu,[4] Shigeru Tsuchiya,[5] Hidetoshi Takada,[6] Toshiro Hara,[6] Nobuaki Kawamura,[7] Tadashi Ariga,[7] Hideo Kaneko,[8] Naomi Kondo,[8] Ikuya Tsuge,[9] Akihiro Yachie,[10] Yukio Sakiyama,[11] Tsutomu Iwata,[12] Fumio Bessho,[13] Tsutomu Ohishi,[14] Kosuke Joh,[14] Kohsuke Imai,[15] Kazuhiro Kogawa,[15] Miwa Shinohara,[16] Mikiya Fujieda,[16] Hiroshi Wakiguchi,[16] Srdjan Pasic,[17] Mario Abinun,[18] Hans D. Ochs,[19] Eleonore D. Renner,[19,20] Annette Jansson,[20] Bernd H. Belohradsky,[20] Ayse Metin,[21] Norio Shimizu,[3] Shuki Mizutani,[2] Toshio Miyawaki,[22] Shigeaki Nonoyama,[15] and Hajime Karasuyama[1]

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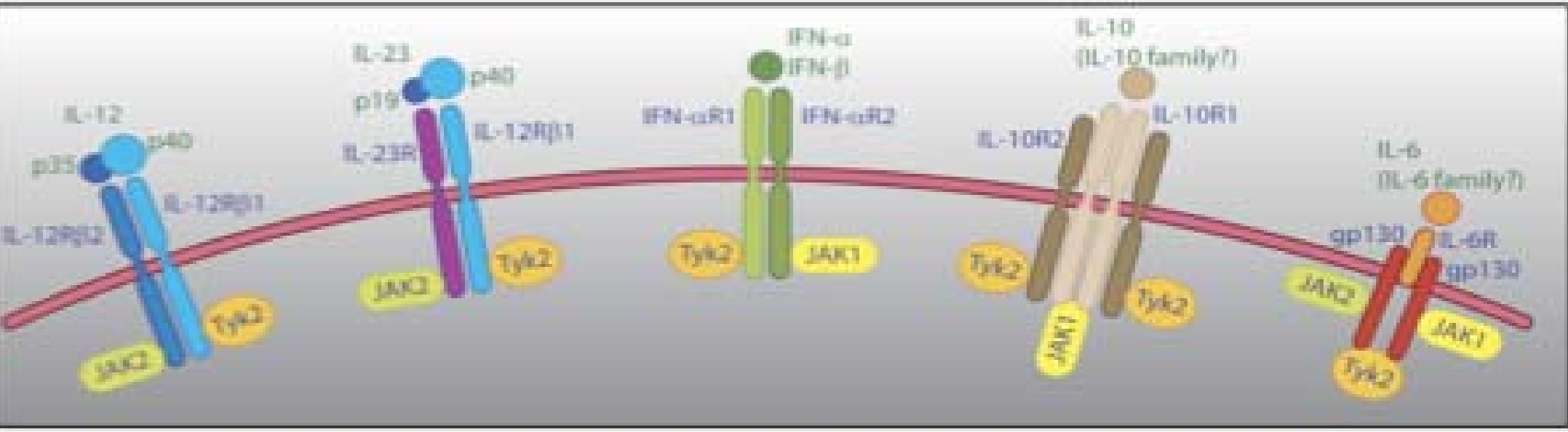
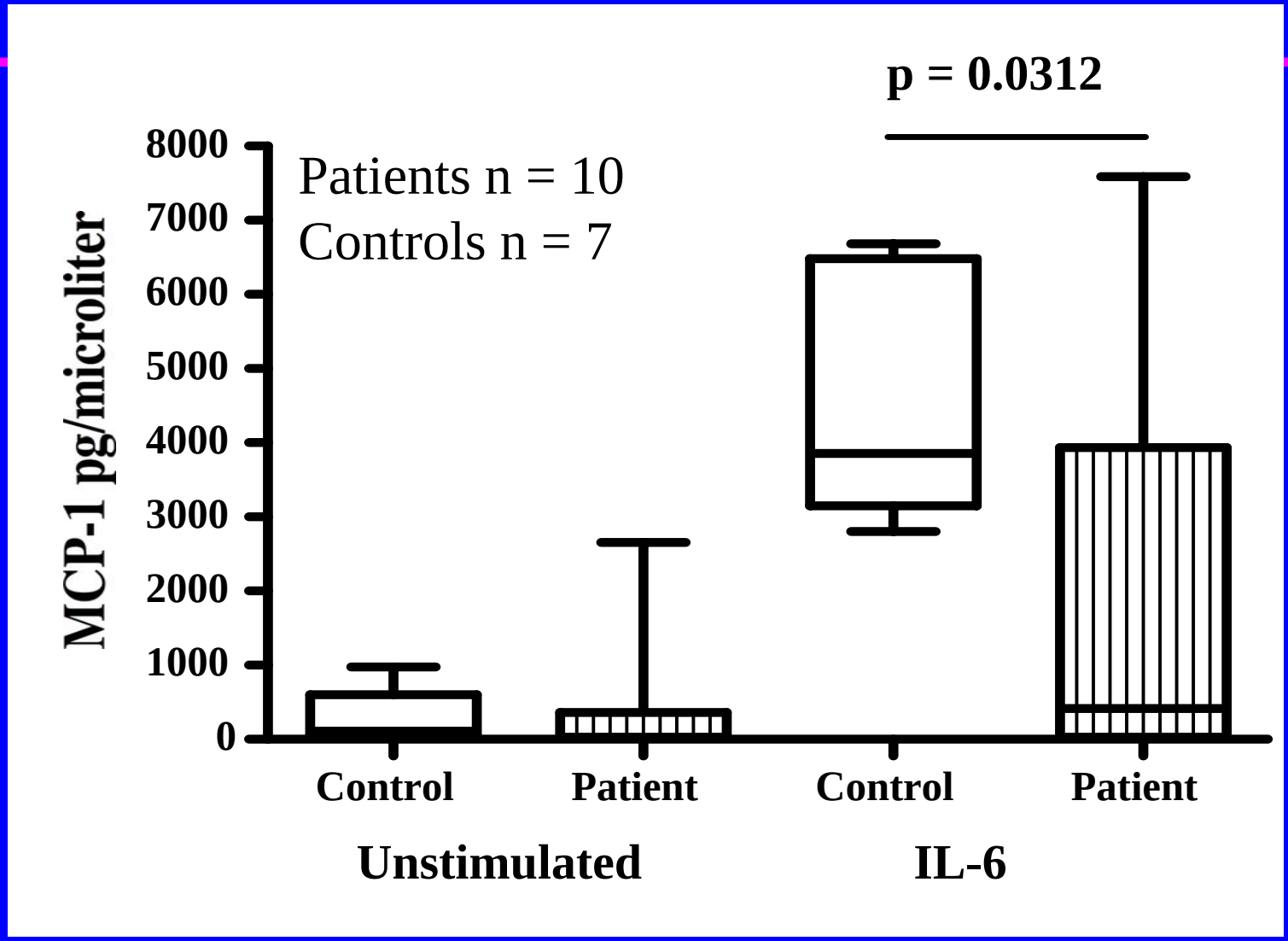


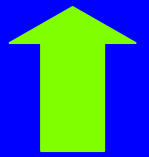
MCP-1 Production is Decreased in HIES: IL-6



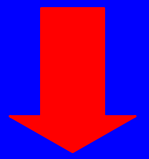
In vitro Phenotyping in HIES

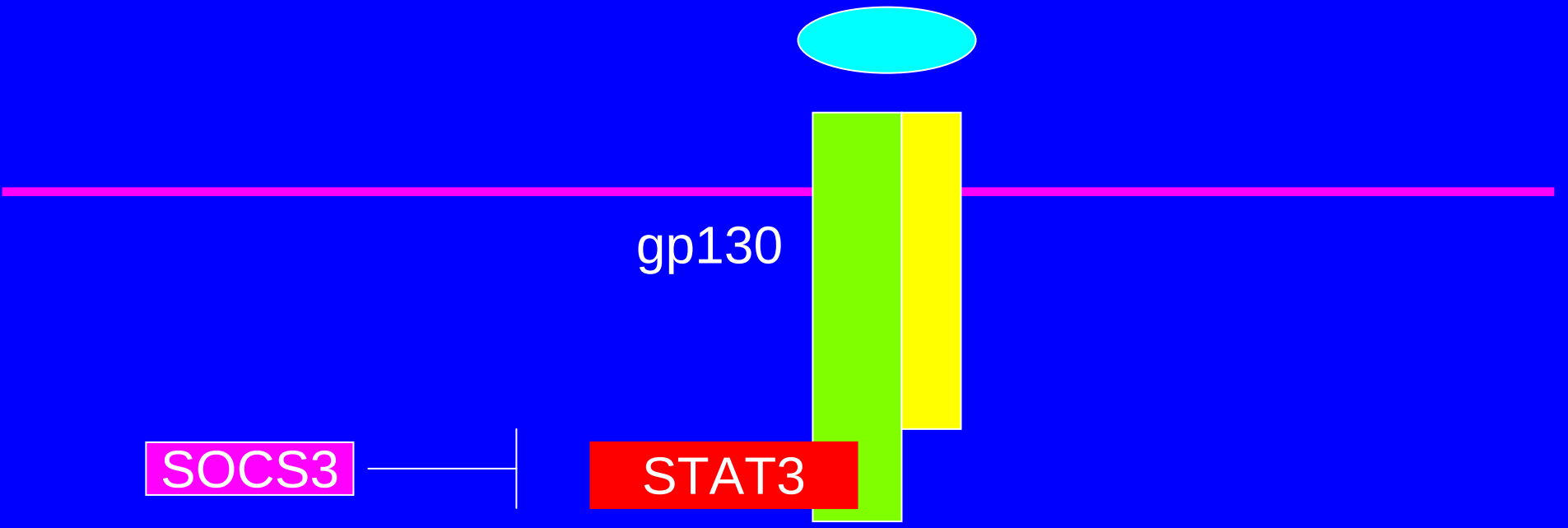
Transcripts of IFN induced genes in unstimulated PMN and PBMC

Production of TNFα, IL-12, and IFNγ in response to multiple stimuli



Production of MCP-1 in response to IL-6



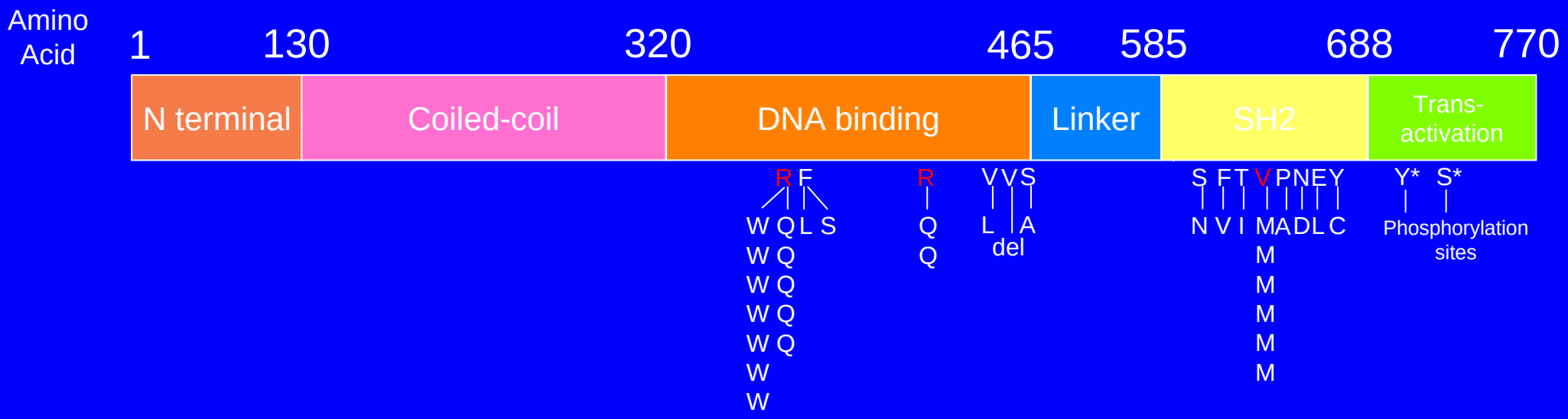


IL-6
IL-10
TGFb
MCP-1

IL-12
TNFa
IFNg
IFNb

ORIGINAL ARTICLE

STAT3 Mutations in the Hyper-IgE Syndrome



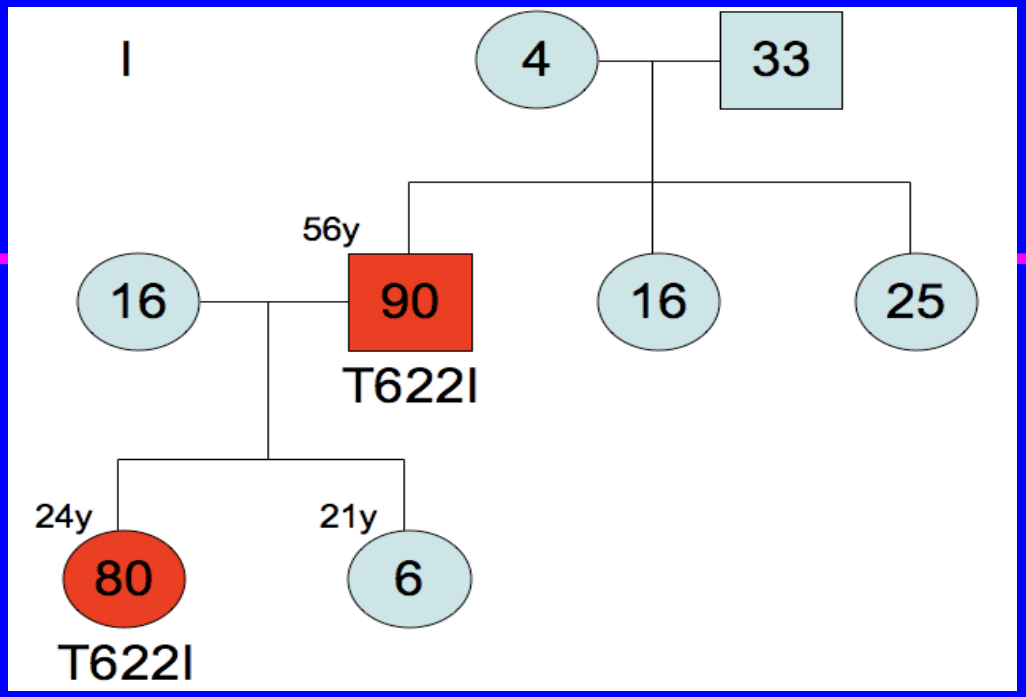
DNA Binding

1144 C->T; R382W
1145 G->A; R382Q
1150 T->C; F384L
1151 T->C; F384S
1268 G->A; R423Q
1381 G->C; V461L
1387 delGTG; V463del
1393 T->G; S465A

SH2 Domain

1832 G->A; S611N
1861 T->G; F621V
1865 C->T; T622I
1909 G->A; V637M
1915 C->G; P639A
1939 A->G; N647D
1954 G->A; E652L
1970 A->G; Y657C

N Engl J Med 2007;357:1608-19.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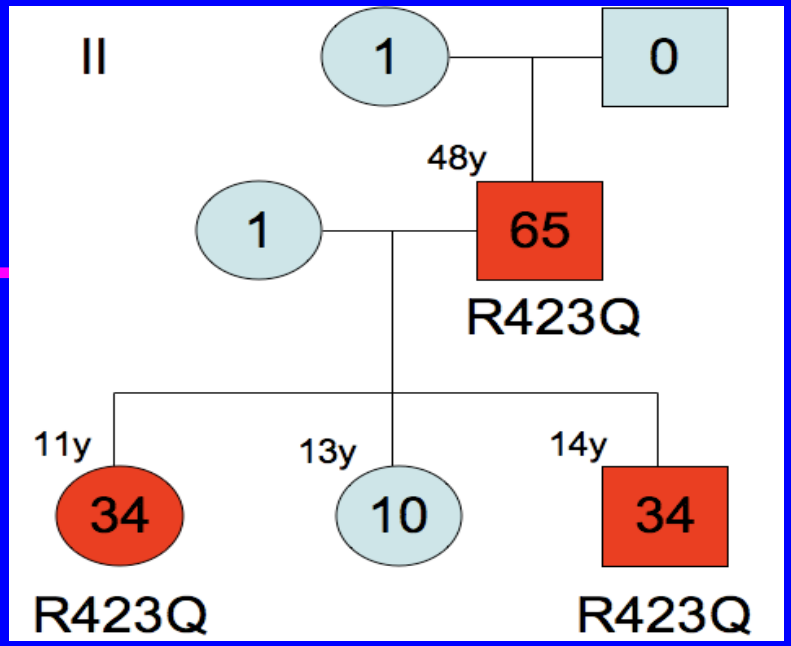
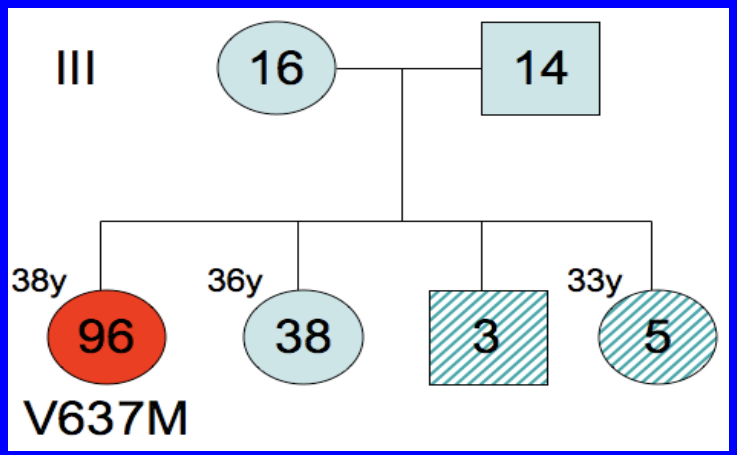
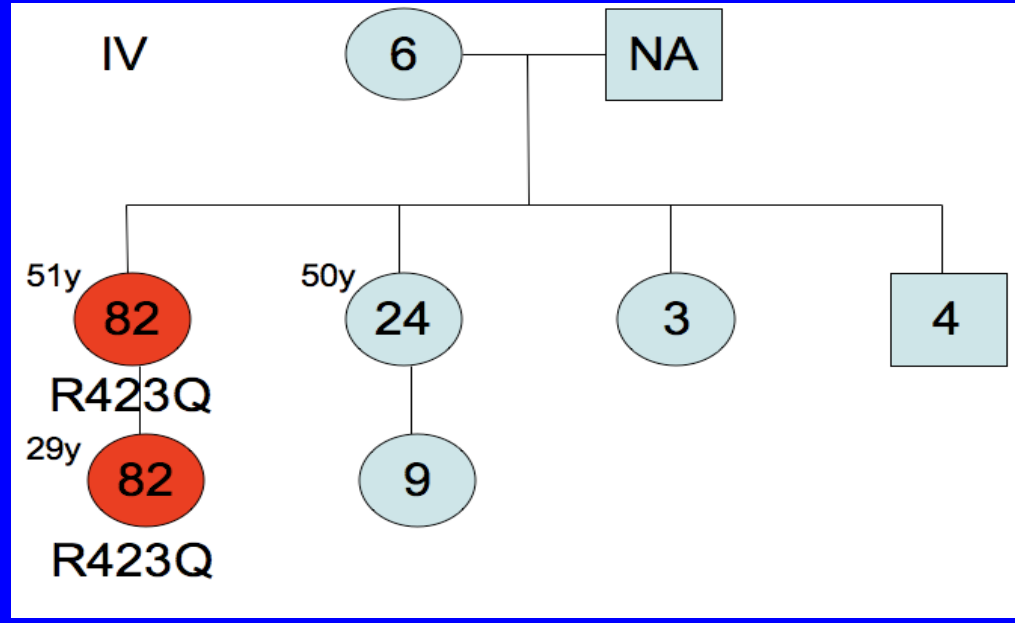
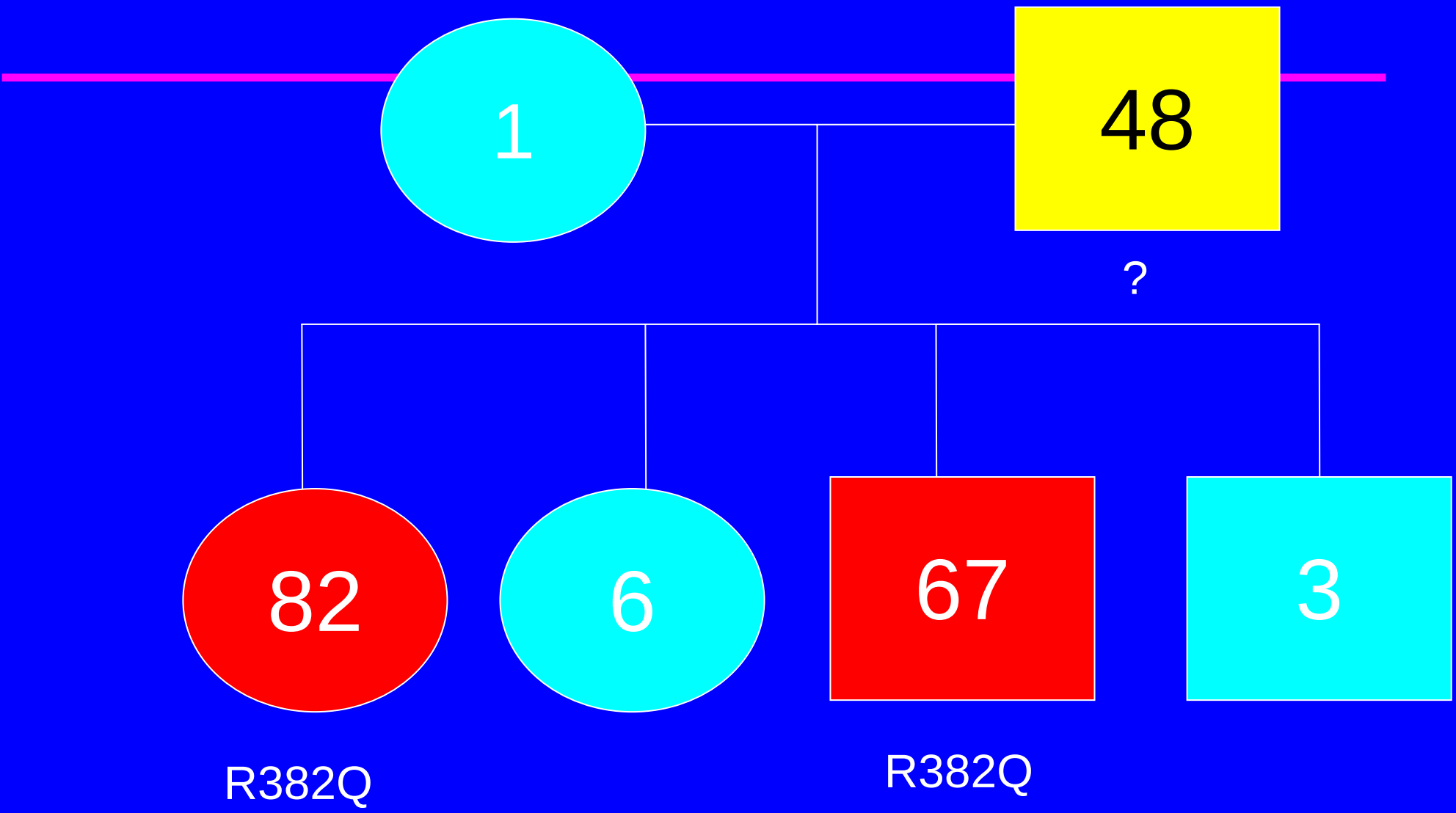
25
T622I
24y
21y
80
6
T622I


II
1
0
48y
1
65
R423Q
11y
13y
14y
34
10
34
R423Q
R423Q


III
16
14
38y
36y
33y
96
38
3
5
V637M


IV
6
NA
51y
50y
82
24
3
4
R423Q
29y
82
9
R423Q


1
48
?
82
6
67
3
R382Q
R382Q

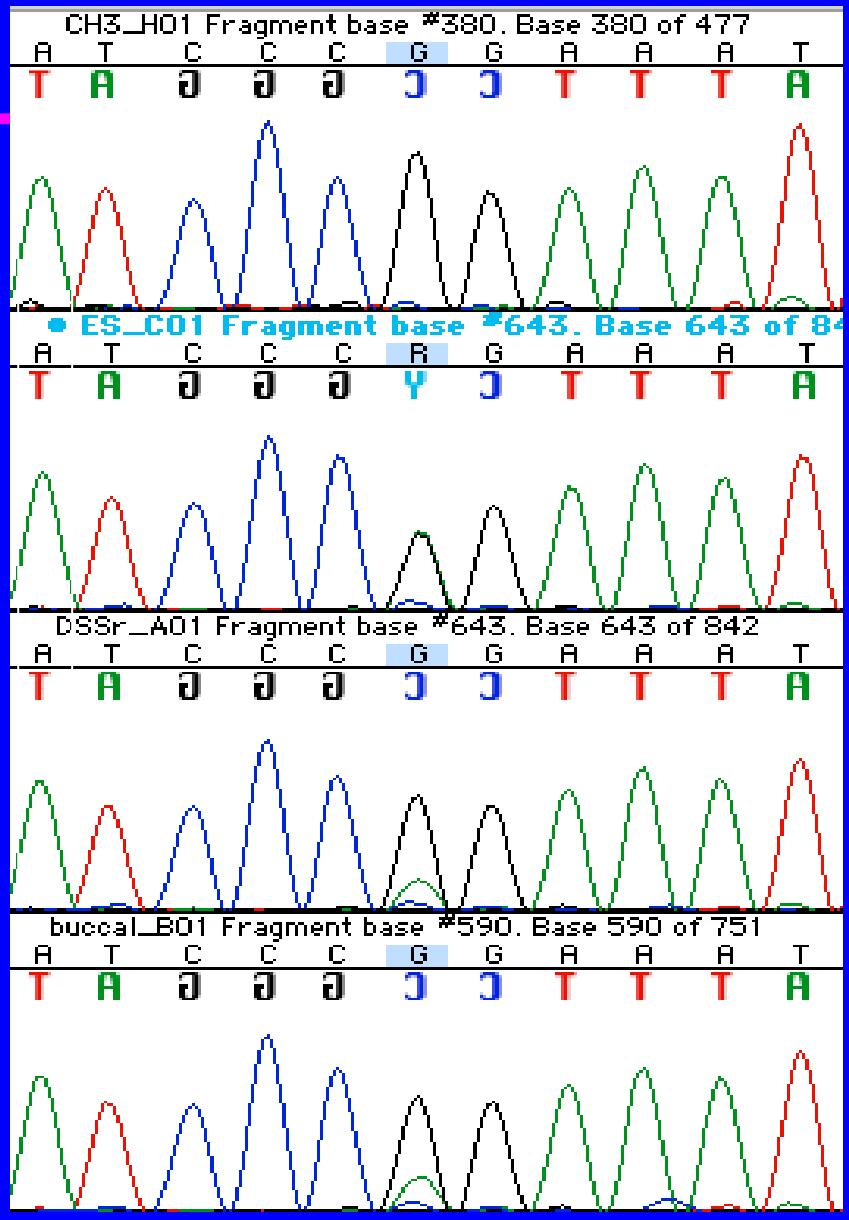
1145 G-A R382Q Hotspot Mutation: Somatic Mosaic

WildType G

Heterozygous G/A

Lymphocytes
Mosaic G/A

Buccal swab
Mosaic G/A



From Mouse to Man?

Mouse

Excessive lung inflammation, airspace dilatation

Eosinophilia

Increased osteoclasts and osteopenia

Cardiac myocyte TNFa production, age-dependent cardiomyopathy

Increased demyelination and astrocytosis

IL-22 dysregulation

Human

Post-infectious lung cysts, bronchiectasis

Eosinophilia

Increased osteoclasts and osteopenia

Coronary artery aneurysms, seen in adults (4/8)

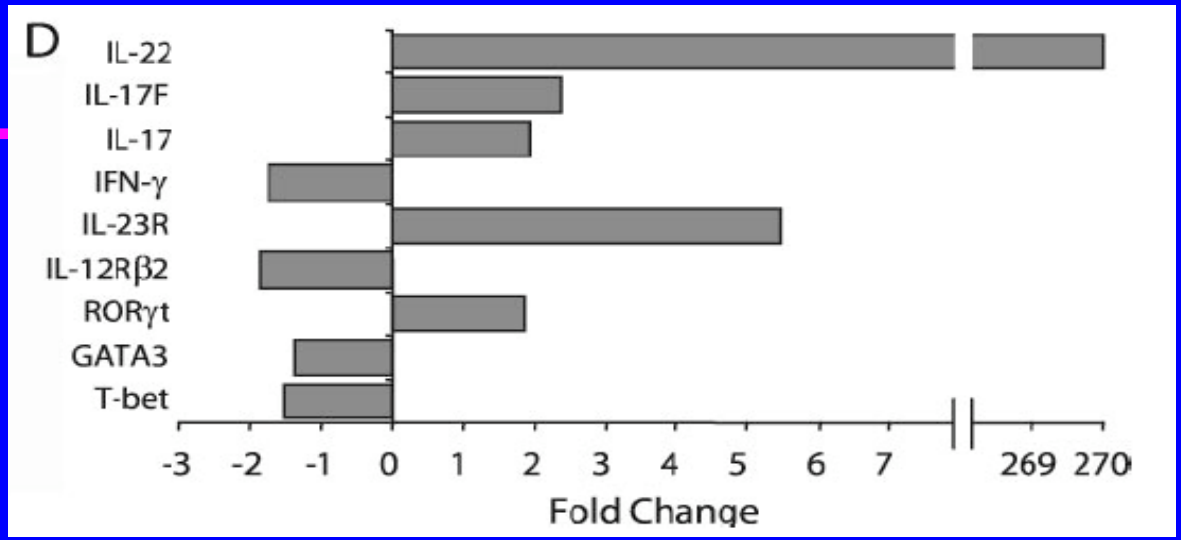
T2 hyperintensities in brain (UBOs)

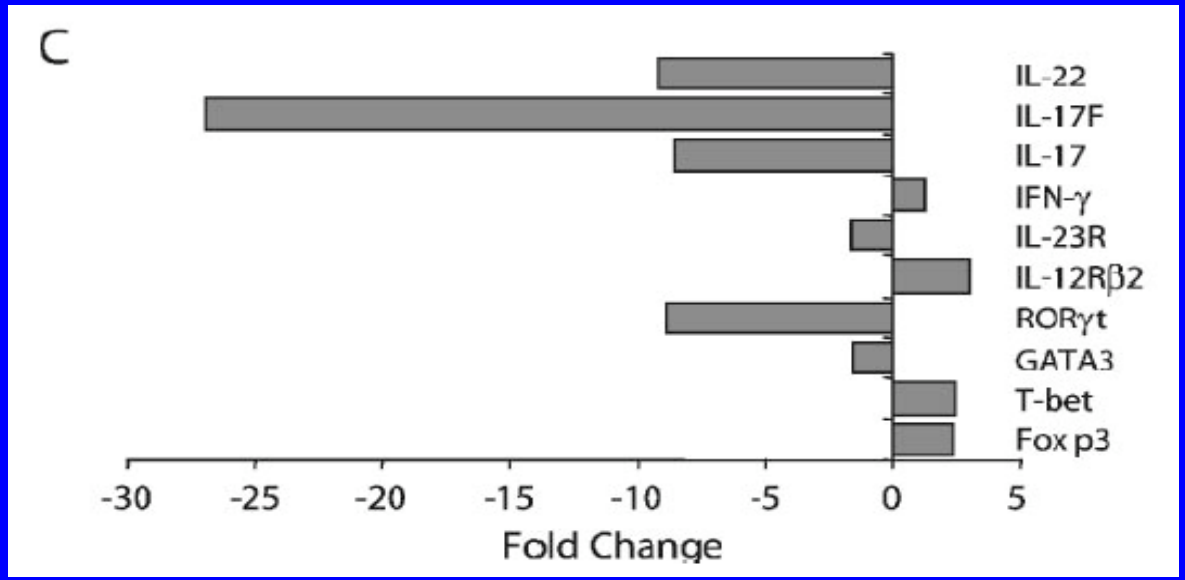
Skin and lung infections

STAT3 and IL-17

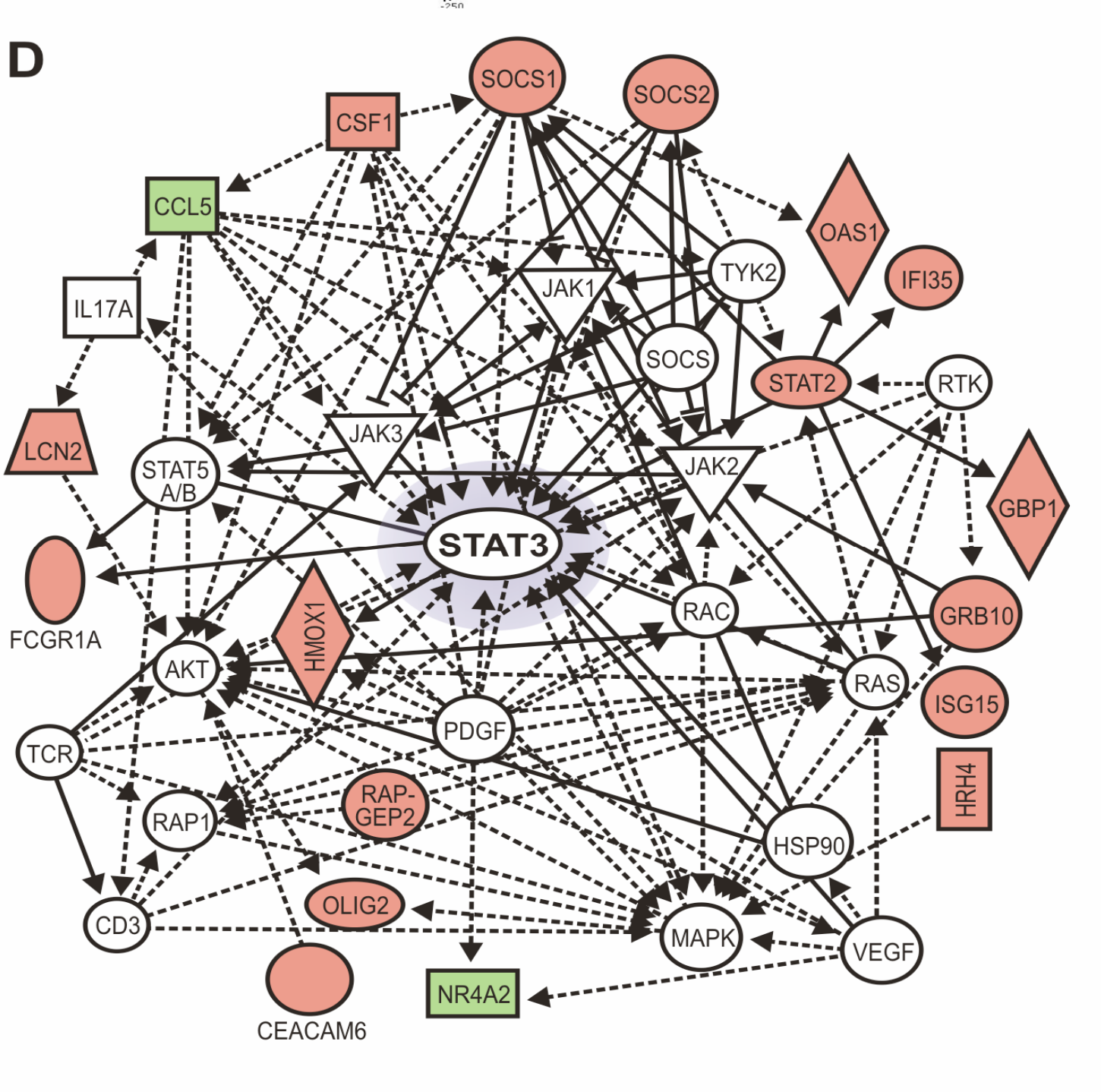
Constitutively Activated STAT3

STAT3 KO





Yang et al J Biol Chem. 2007;282:9358-63.


D
SOCS1
SOCS2
CSF1
CCL5
OAS1
IFI35
TYK2
JAK1
IL17A
SOCS
STAT2
RTK
LCN2
JAK3
JAK2
STAT5
A/B
GBP1
STAT3
FCGR1A
HMOX1
RAC
GRB10
AKT
RAS
ISG15
PDGF
TCR
HRH4
RAP-
GEP2
RAP1
HSP90
OLIG2
CD3
MAPK
VEGF
NR4A2
CEACAM6

Still Unexplained

- The IgE elevation (IL-21?)
- The specificity of infections, including *Pneumocystis*, histoplasmosis, cryptococosis, candida (IL-17?, IL-22?)
- The facies
- The voice
- How can we use this to change outcome

Conclusions: *STAT3*

Heterozygous mutations underlie both dominant and sporadic human HIES

The mutations cluster in two highly conserved domains

Mutations result in dysregulated cytokine production and signaling

Clinical scores correlate with mutation status

Aspergillus causes fatal localized and metastatic complications

Therefore, the patients probably have normal " systemic " resistance to staphylococcal infections. The second observation is that the abscesses are cold. Miles (1956) has shown that the first few minutes after bacteria have been injected into the skin constitutes the decisive period for inflammation. Local nonspecific factors are probably of primary importance during the decisive period. We suggest that these patients have a defect in local non-specific mechanisms of resistance. Perhaps there is an abnormality of the mediators of the acute inflammatory response (Spector 1964).

The pitiful appearance of these patients and the history of recurrent abscesses and skin infections makes the name " Job's syndrome " seem suitable.

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