Predicting the unpredictable

Individual risk stratification of patients at risk of IA

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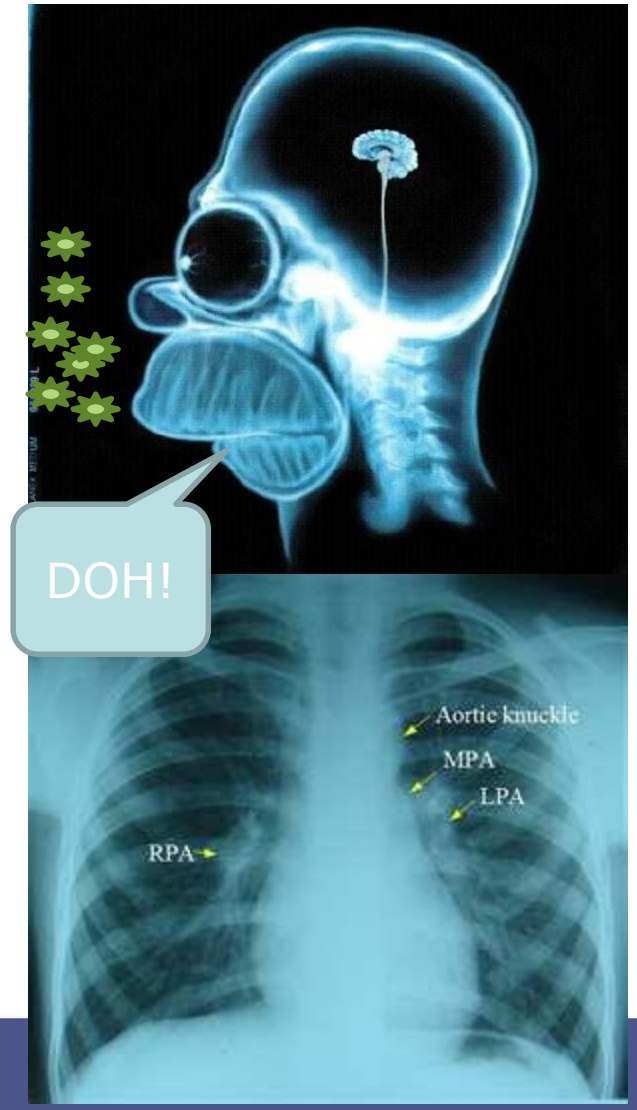
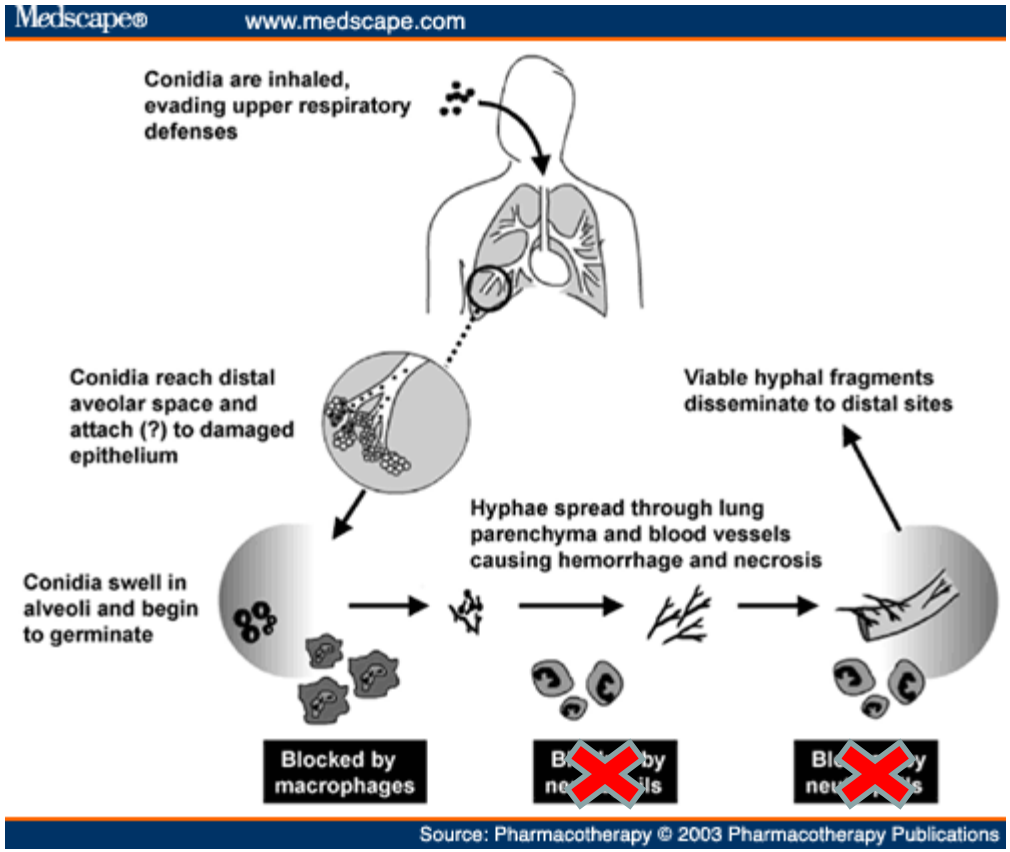
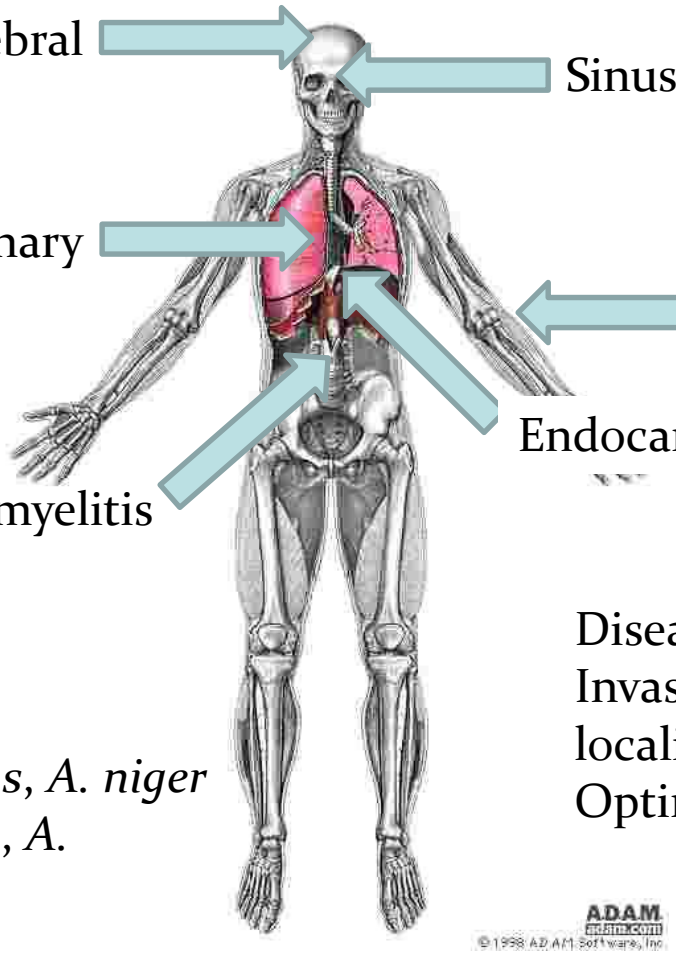
Invasive Aspergillosis – Disease process





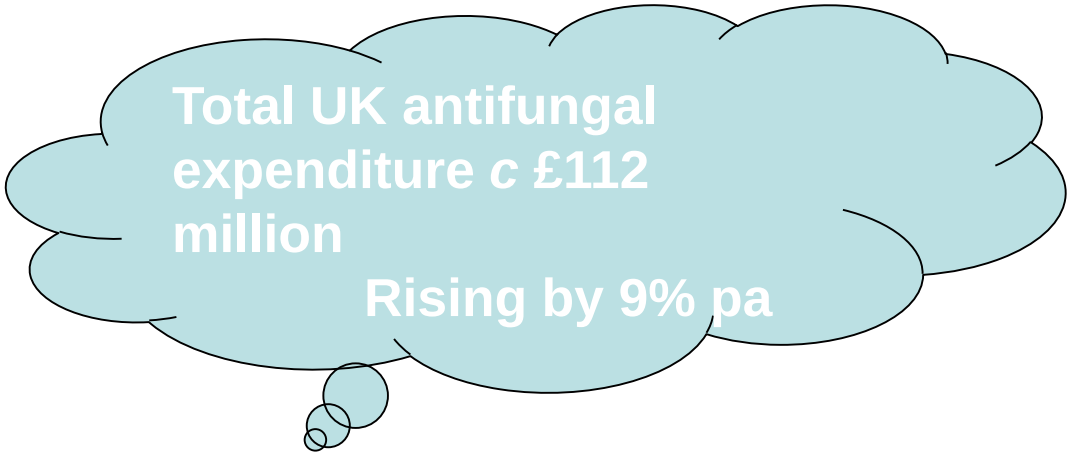
Aspergillosis – Disease spectrum



Common species:
A. Fumigatus, A. flavus, A. niger
A. terreus, A. nidulans, A. versicolor

Disease presentation,
Invasive, non-invasive, localised, allergic
Optimal Specimen type

Problem



- Antifungal expenditure is completely out of proportion with the scale of the problem
- Incidence of IFD in ICU (candida) <0.6%
- Aspergillus infection in haematological malignancy (0.5-12%)
- Aspergillus in SOT <5%

Harrison D et al Fungal Infection Risk Evaluation (FIRE) Study. *Health Technol Assess* 2013; 17(3).
Pagano L et al. Haematologica 2006; 91: 1068-1075
Pagano L et al. Clin Infect Dis 2007; 45: 1161-1170

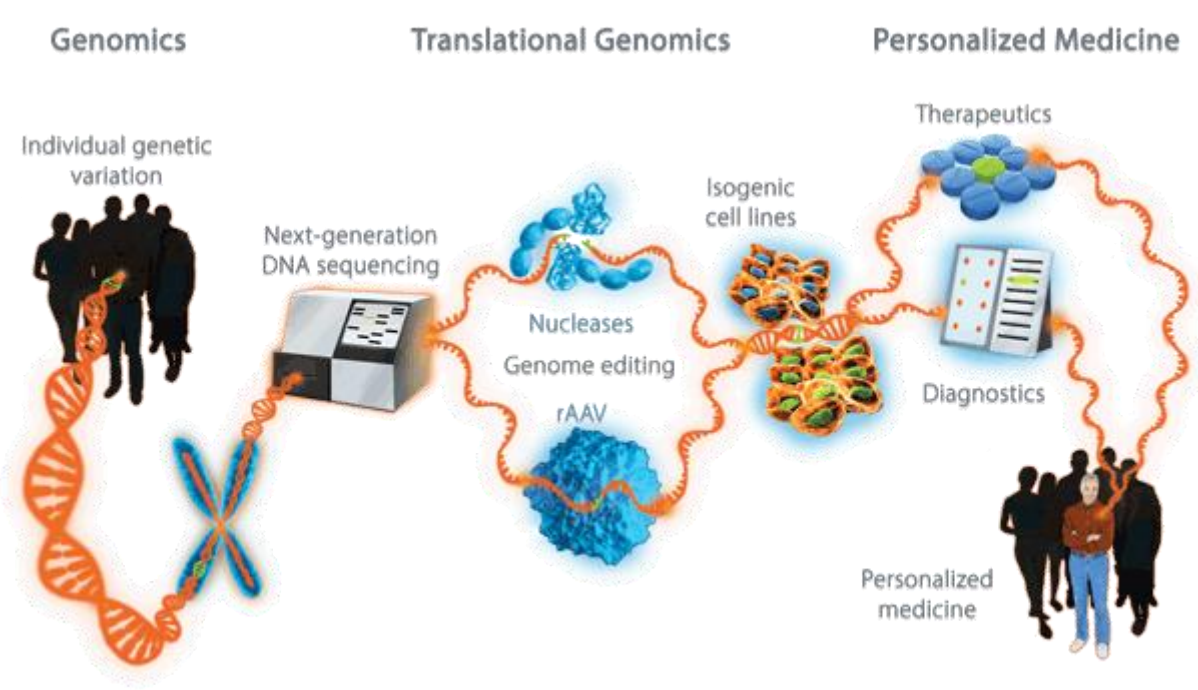
Reasons

- Infection associated with significant morbidity and mortality
- Signs and symptoms of systemic infection are nonspecific
- Delays in treatment associated with poorer outcome
- Conventional diagnostic techniques are suboptimal

Novel Diagnostic accuracy

- Screening by PCR AND GM EIA can enable a diagnosis of IA to be excluded
  - Both negative: Post test probability of no IA: 99.6%
- Multiple positive PCRs and EIAs can be used to accurately diagnosis IA
  - Both multiple positive: Post test probability of IA: 54.5%
  - Pre test probability: 9.7%
- Biomarkers are earliest markers in 85% of cases
  - Dependent on the timing of presentation of the patient
  - Frequency of testing
  - AFT
- Use anti-fungals more cost effectively
  - Reduction of £500K per annum

Barnes *et al.* 2013



Predicting the unpredictable

Stratify haematology patients according to:

1) Clinical risk
2) Genetic risk
3) Mycological evidence

Develop a predictive for model for individual patient management

Study population

- 322 haematology patients
- 54 patients with proven/probable IA
- 268 patients with NEF
- 225 patients with evidence not meeting EORTC/MSG criteria excluded

- Incidence of IA: 16.7%

Identifying clinical risk

TABLE 1 Patient demographics, underlying diseases, hematopoietic SCT status, biomarker (PCR) positivity, and additional viral infections stratified by IA classification

Parameter	Proven IA (n = 6)	Probable IA (n = 48)	Possible IA (n = 20)	NEF (n = 268)	P value[f]
Male/female ratio	2:1	1.7:1	1.9:1	1.5:1	0.7667
Median age (yr)	58	52	64	61	0.31[g]
No. with underlying disease					
AL/MDS	3	25	15	91	**0.011**
Lymphoma	1	13	3	90	0.3389
Myeloma	1	4	0	50	0.1134
Chronic leukemia	1	4	2	18	0.5605
Other[a]	0	2	0	19	0.5521
Mortality rate (%)	66.7	54.2	60.0	45.5	0.1302
Transplant rate (%)					
Combined	50.0	58.3	20.0	33.6	**0.0018**
Allogeneic SCT	50.0	45.8	20.0	9.3	**<0.0001**[h]
Autologous	0.0	12.5	0.0	24.3	**0.00321**
No transplant	50.0	41.7	80.0	66.4	**0.0018**
No. with GVHD	2	12	2	9	0.2757[i]
No. PCR positive					
1 positive threshold[b]	6	44	15	93	**<0.0001**
≥2 positive threshold[c]	6	36	10	39	**<0.0001**
No. with additional infection(s)					
CMV	4	11	3	22	0.3494[j]
Respiratory virus	1	34	4	38	**0.0025**[k]
Influenza A or B virus	0	9	1	11	0.2113[k]
Parainfluenza	1	7	1	8	0.0988[k]
Rhinovirus	0	5	0	10	0.4879[k]
RSV	0	12	2	9	**0.0046**[k]
Adenovirus	0	1	0	0	0.2996[k]
Multiple respiratory viruses	0	8[d]	0	8[e]	0.1483[k]

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Identifying genetic risk

- Genomic DNA was a bi-product extracted from whole blood as part of routine PCR testing
- Genotyping for the polymorphisms was performed using Taqman single-nucleotide assays on the 7500 ABI real-time PCR system

SNP rs number	gene	Location	Nucleotide sudstitution	Polymorhism association	Biological effect
Rs16910526	Dectin-1	Exon	A/C	Early stop codon	Candida colonization CMC, ?aspergillosis
Rs7309123	Dectin-1	Intron	C/G	Unknown	?increase IPA
Rs11465384	DC-SIGN	3'-UTR	A/G	Affects RNA expression	?increase IPA
Rs7248637	DC-SIGN	3'-UTR	A/G	Affects RNA expression	?increase IPA
Rs7252229	DC-SIGN	intron	C/G	Unknown	?increase IPA

Sainz et al PLOS One 2011

SNP's associated with IA

Gene	Gene locus	ABI-Assay	Proven/Prob IA	NEF	Odds-ratio (95% CI)	*P* value
Dectin-1	rs16910526	C_33748481_10	12/54	51/268	1·2 (0·6-2·6)	0·576
Dectin-1	**rs7309123**	**C_3130832_10**	**47/54**	**173/268**	**3·7 (1·5-9·3)**	**0·001**
DC-SIGN	**rs11465384**	**C_25996399_10**	**15/54**	**30/268**	**3·1 (1·4-6·5)**	**0·004**
DC-SIGN	rs7252229	C_29620333_10	17/54	55/268	1·8 (0·9-3·6)	0·106
DC-SIGN	**rs7248637**	**C_29710787_10**	**18/54**	**37/268**	**2·9 (1·4-5·9)[a]**	**0·001[a]**
Combination	**Haplotype and rs7309123**	**C_3130832_10 C_25996399_10 C_29710787_10**	**20/54**	**31/268**	**4.5 (2.2-9.2)**	**<0.001**

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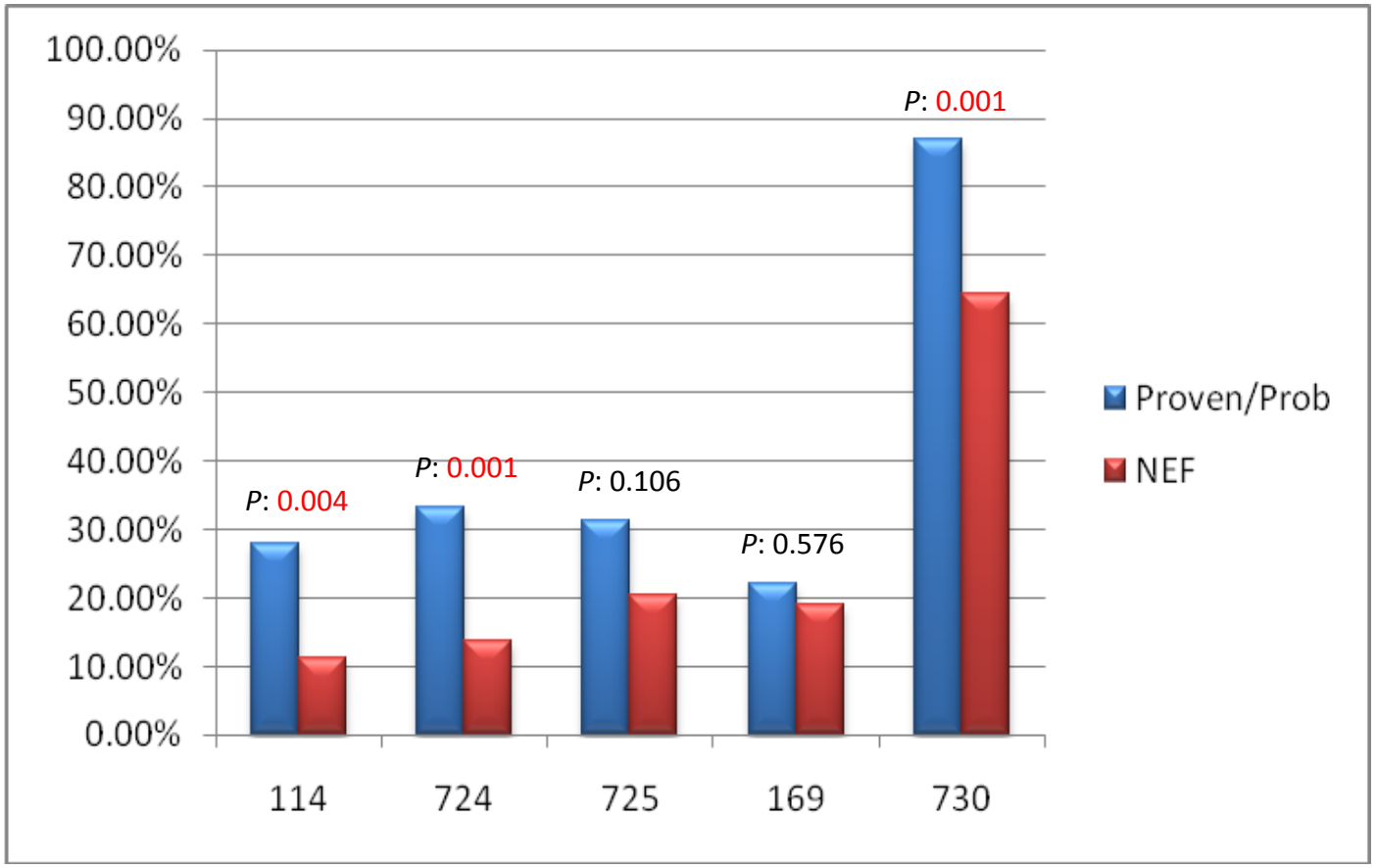
SNP's associated with IA



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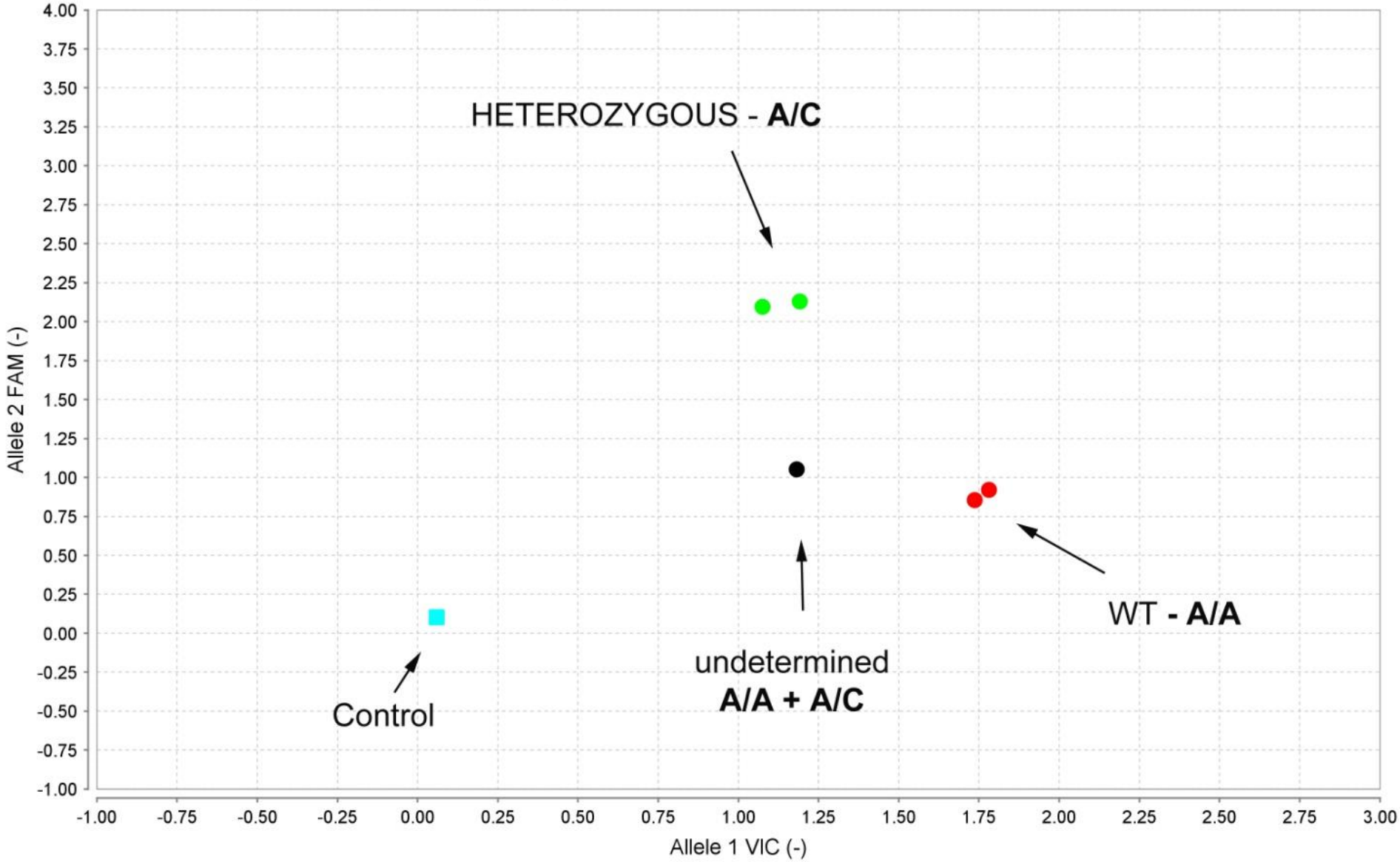
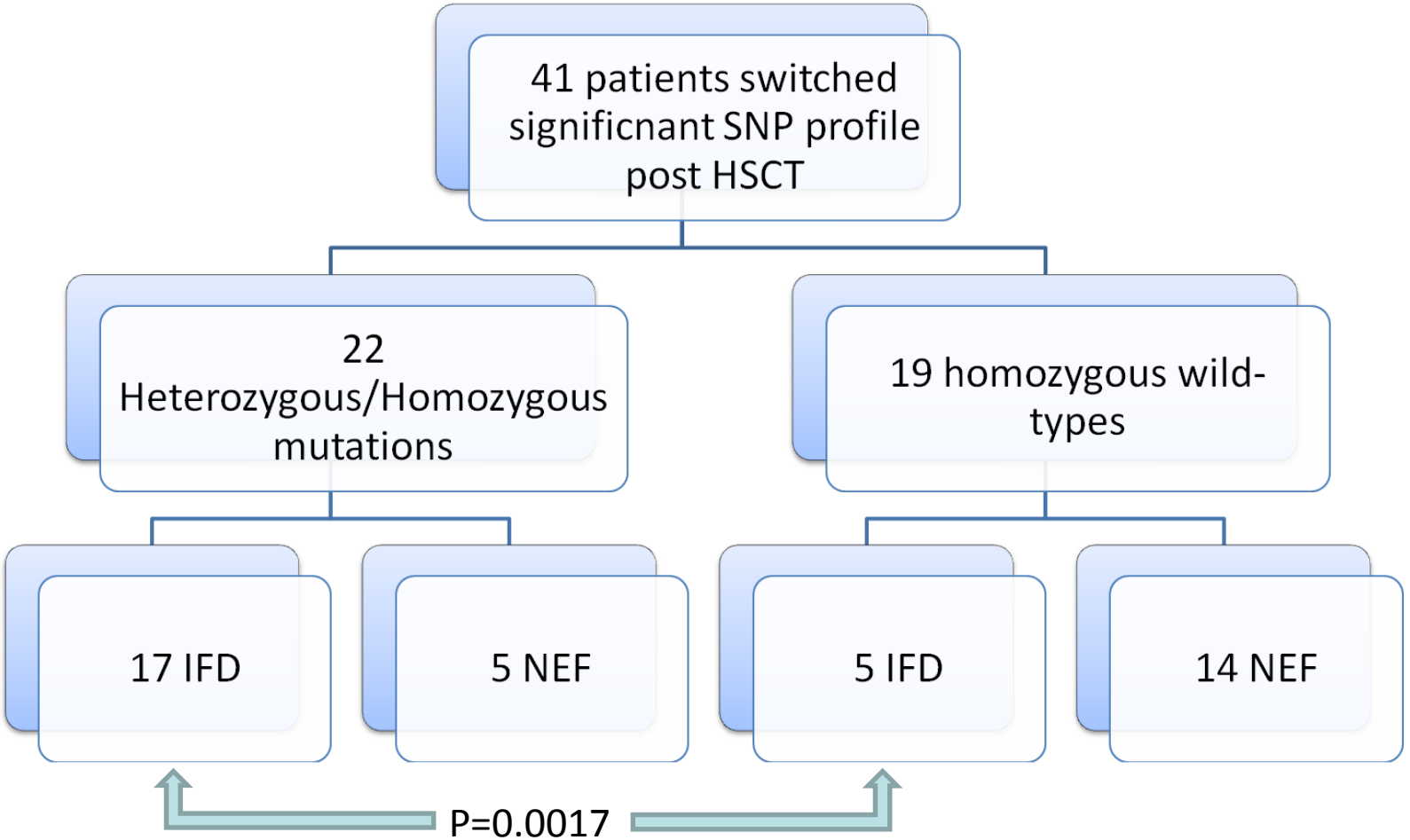
Dectin-1 SNP (rs169) Genotyping:

Introduction of Polymorphism following bone marrow transplant (BMT) in patient that went on to develop IA.



Genotype switching post HSCT



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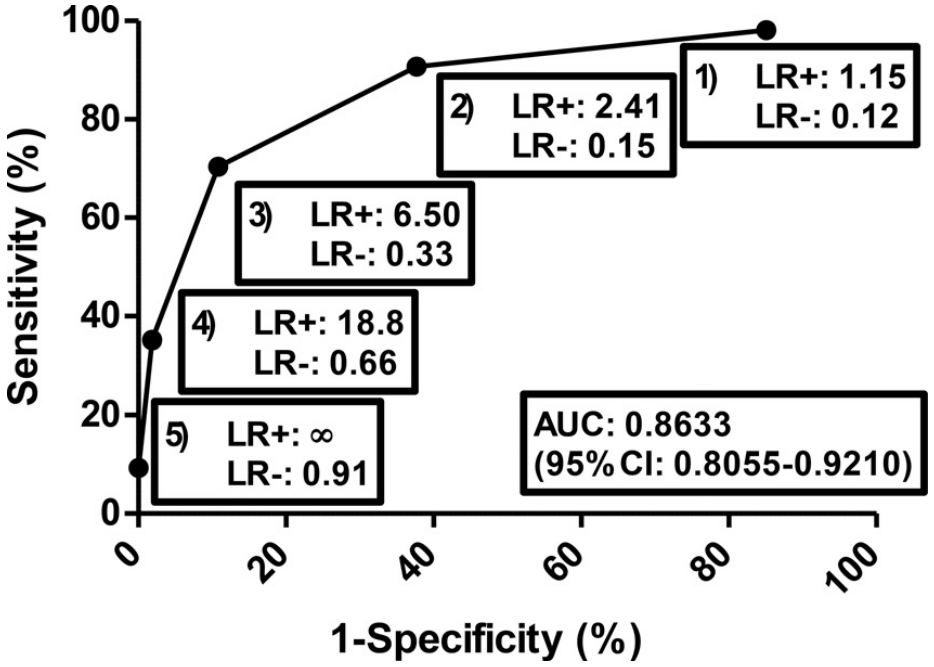
Final Model



- Final Risk Variables:
  - Allogeneic SCT
  - SNP in Dectin 1 rs7309123
  - SNP in DC-SIGN Haplotype
  - Aspergillus PCR positivity
  - Respiratory virus infection

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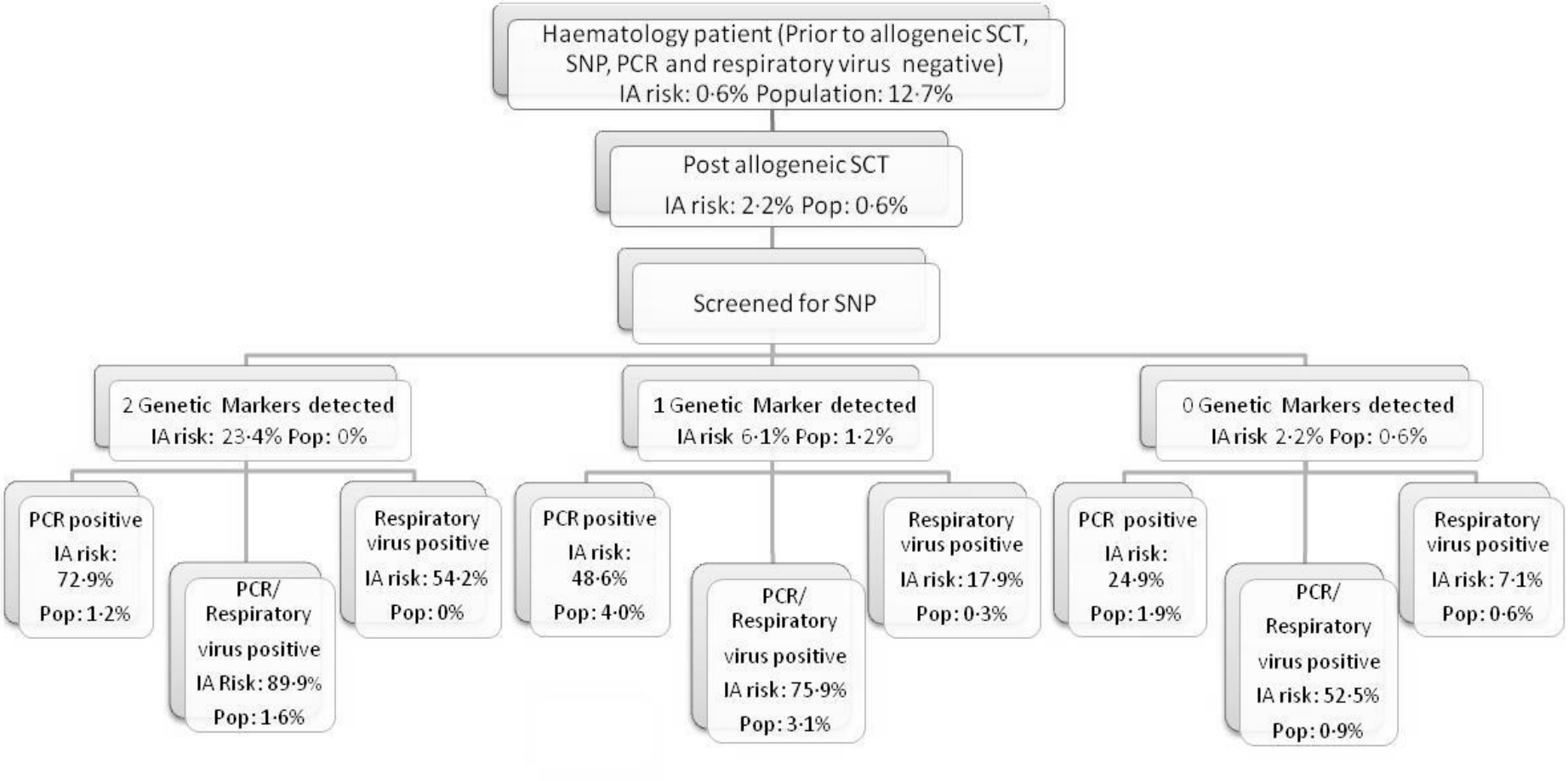
Predicting IA in BMT



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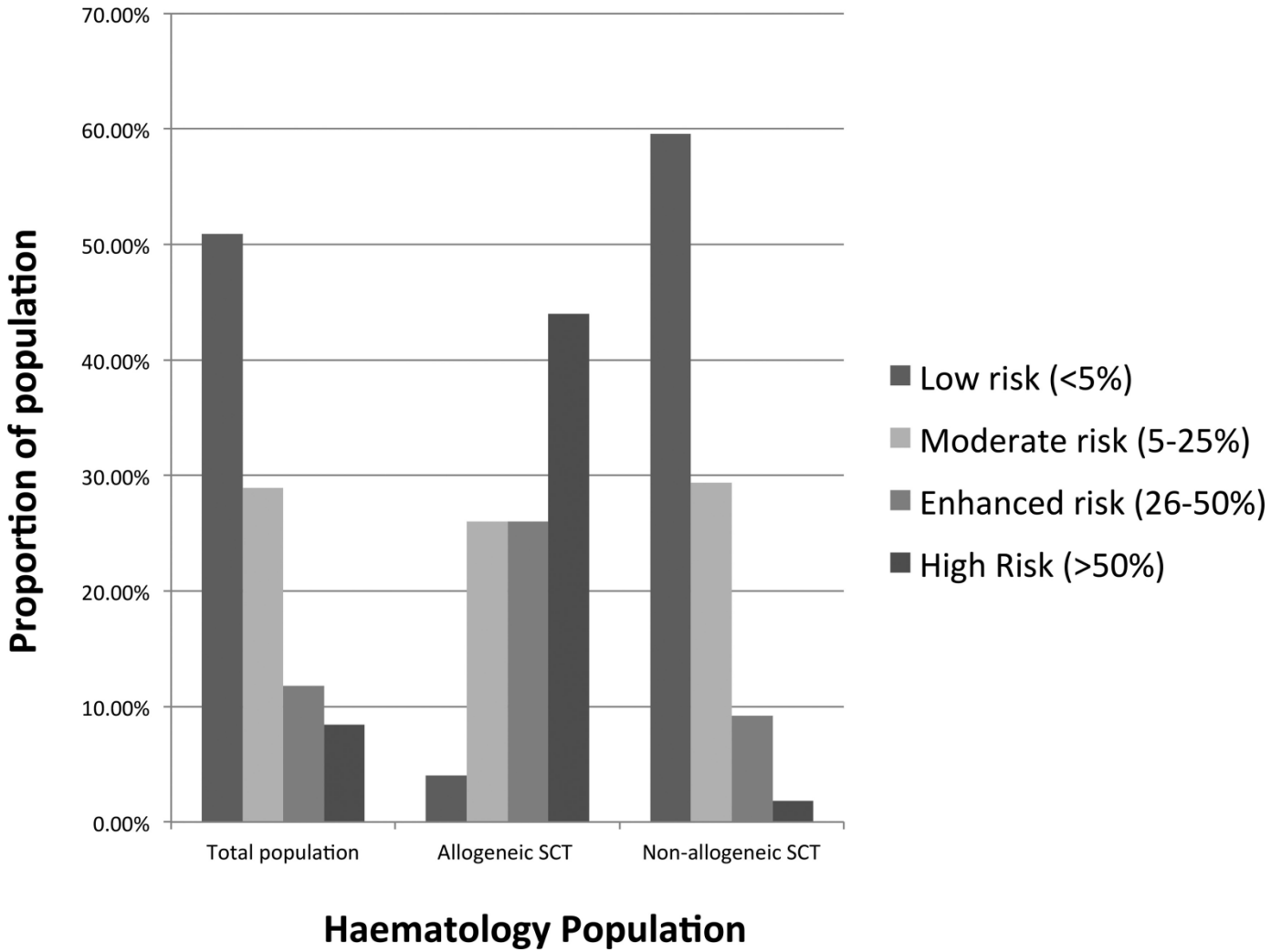
Breakdown of risk



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Summary

- Previously determined clinical risk factors remain
- Three independent SNPs identified that may influence risk of invasive aspergillosis
- Combining clinical and genetic risk factors may further improve diagnostic strategies
- Screening patients/potential donors is quick and cheap
- Results can inform risk stratification and guide diagnostic/prophylactic strategies
- Personalised medicine
- Improve prescribing accuracy
- Need to automated computation of predicted risk

Acknowledgements

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