Etiology of fever and role of T cells in Gram-negative sepsis: Dhaka experience

Fazle Rabbi Chowdhury

FCPS (Medicine), MSc. (Infectious Disease, UK)
PhD in Clinical Medicine (Oxford, UK)

Assistant Professor, Internal Medicine
Bangabandhu Sheikh Mujib Medical University, Dhaka

- Bacterial and viral infectious diseases are still the leading cause of death in Southeast Asia, comparable to injuries, cardiovascular diseases and other non-communicable diseases

- The etiology of febrile illness remains poorly characterized in many places in the developing world

- The causative pathogens are not usually identified so patients are usually treated empirically with antibiotics

- Although the burden of some infections is believed to be substantial (e.g. enteric fever), the prevalence of many others, is less known and frequently under-diagnosed

- Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (bacterial, viral and fungal)

- It is a common and serious problem in both developed and developing countries often requires ICU admission

- The definition of sepsis was modified and updated following publication of the Sepsis-3 in 2016 where the concept of SOFA and q SOFA is introduced. The previous definition was based on SIRS definition (Temp; HR; RR and WBC count)

The global epidemiology and mortality data of sepsis varied between regions mostly because of differences in the aetiology of sepsis, AMR pattern, provision of public health services, availability of ICU facilities, economic status and others

BMJ

RESEARCH

Management of severe sepsis in patients admitted to Asian intensive care units: prospective cohort study

Jason Phua, consultant,[1] Younsuck Koh, professor,[2] Bin Du, professor,[3] Yao-Qing Tang, professor,[4] Jigeeshu V Divatia, professor,[5] Cheng Cheng Tan, consultant,[6] Charles D Gomersall, professor,[7] Mohammad Omar Faruq, professor,[8] Babu Raja Shrestha, consultant,[9] Nguyen Gia Binh, consultant,[10] Yaseen M Arabi, associate professor,[11] Nawal Salahuddin, associate professor,[12] Bambang Wahyuprajitno, consultant,[13] Mei-Lien Tu, respiratory therapist,[14] Ahmad Yazid Haji Abd Wahab, consultant,[15] Akmal A Hameed, consultant,[16] Masaji Nishimura, professor,[17] Mark Procyshyn, respiratory therapist,[18] Yiong Huak Chan, biostatistician[19] for the MOSAICS Study Group

- Population-based data on the prevalence of sepsis in Asia is absent
- **MOSAIC**: 16 SEA/SA and 150 ICU: prevalence was 10.8% in Bangladesh with a overall mortality of 44.5%

Bangladesh Crit Care J March 2013; 1: 8-17

Original Article

Implementation of Sepsis Bundles in Intensive Care Units of Bangladesh: A Prospective Observational Study

Mohammad Omar Faruq[1], ASM Areef Ahsan[2], Mirza Nazim Uddin[3], U H Shahera Khatun[4], Md Abdul Mannan[5], Rownak Jahan Tamanna[6], Kaniz Fatema[7], Fatema Ahmed[8], ARM Nooruzzaman[9], Mohammed Maniruzzaman[10], AKM Shafiqur Rahman[11], AK Qumrul Huda[12], Lutful Aziz[13], Md Sayedul Islam[14], Mohammed Faruk[15], MHM Delwar Hossain[16], Raghib Manzoor[17], SM Hossain Shahid[18], Md Nurul Amin[19]

This prospective study in 15 ICUs in Dhaka identified 10.8% of severe sepsis patients among total admission and mortality was 49.2%

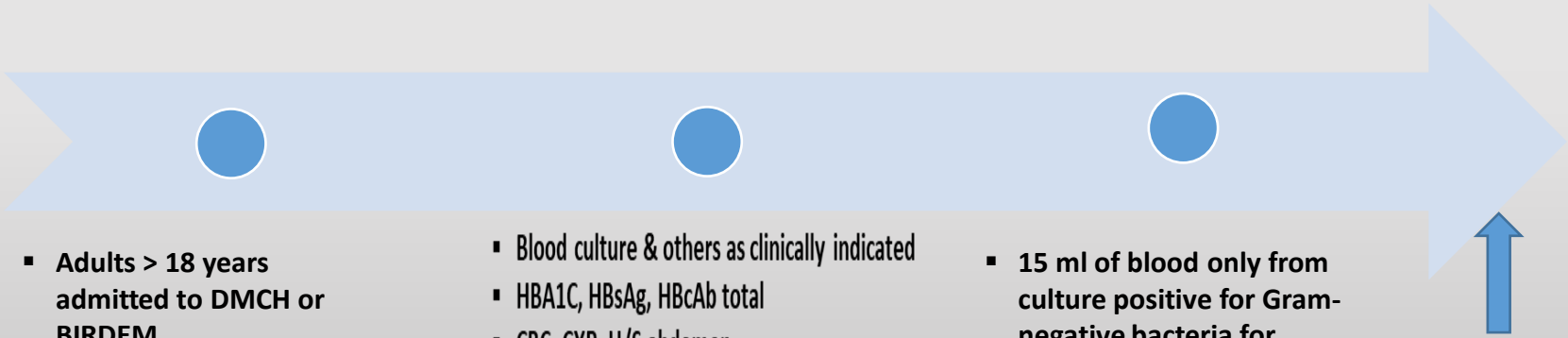
Articles

Causes and outcomes of sepsis in southeast Asia: a multinational multicentre cross-sectional study

Southeast Asia Infectious Disease Clinical Research Network*

- The causative organism of adult sepsis has regional diversity
- Bacterial infections are the commonest cause of adult sepsis in both HIC and LMIC
- In South and South-East Asia, two thirds of adult bacterial sepsis is due to Gram-negative bacteria.

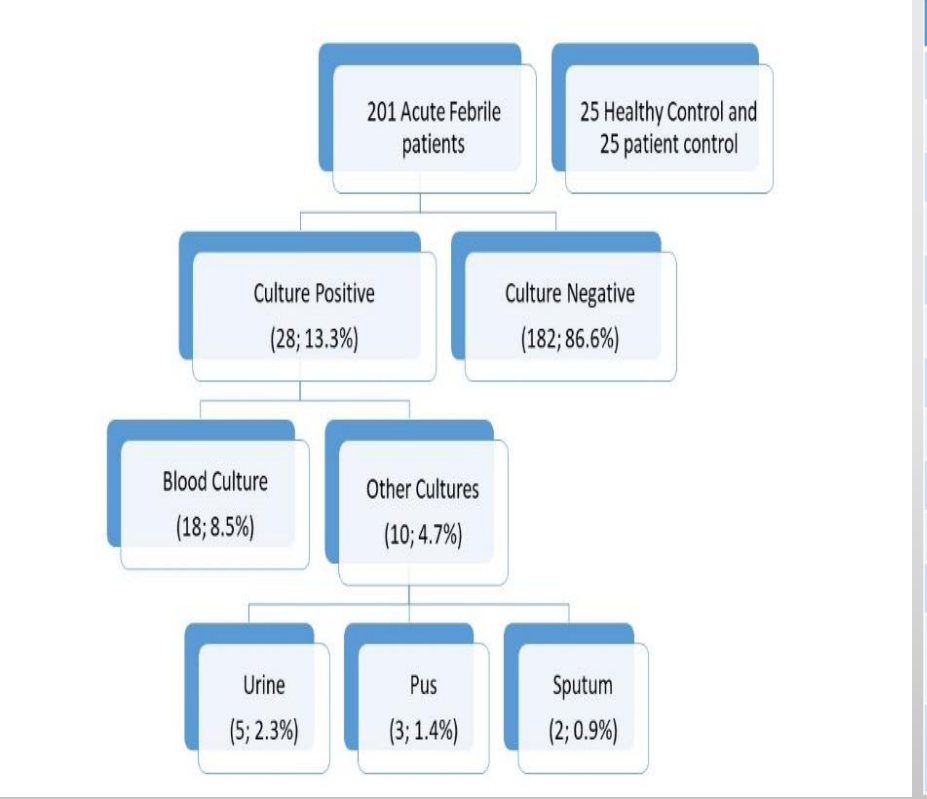
Methods



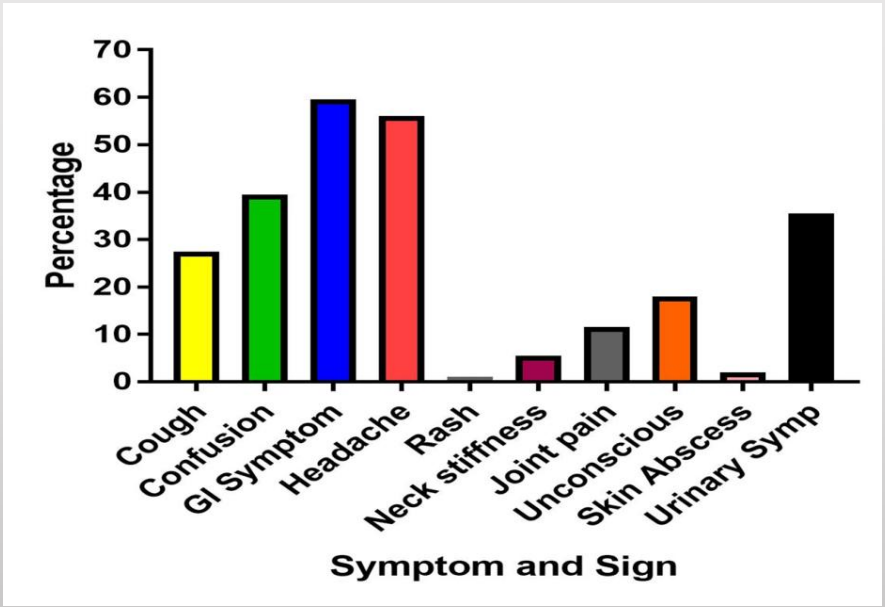
- **Adults > 18 years admitted to DMCH or BIRDEM**
- **Fever >38 degrees for > 48 hours**

- Blood culture & others as clinically indicated
- HBA1C, HBsAg, HBcAb total
- CBC, CXR, U/S abdomen
- NS1 and IgM for Dengue
- PCR and IgM for Chick

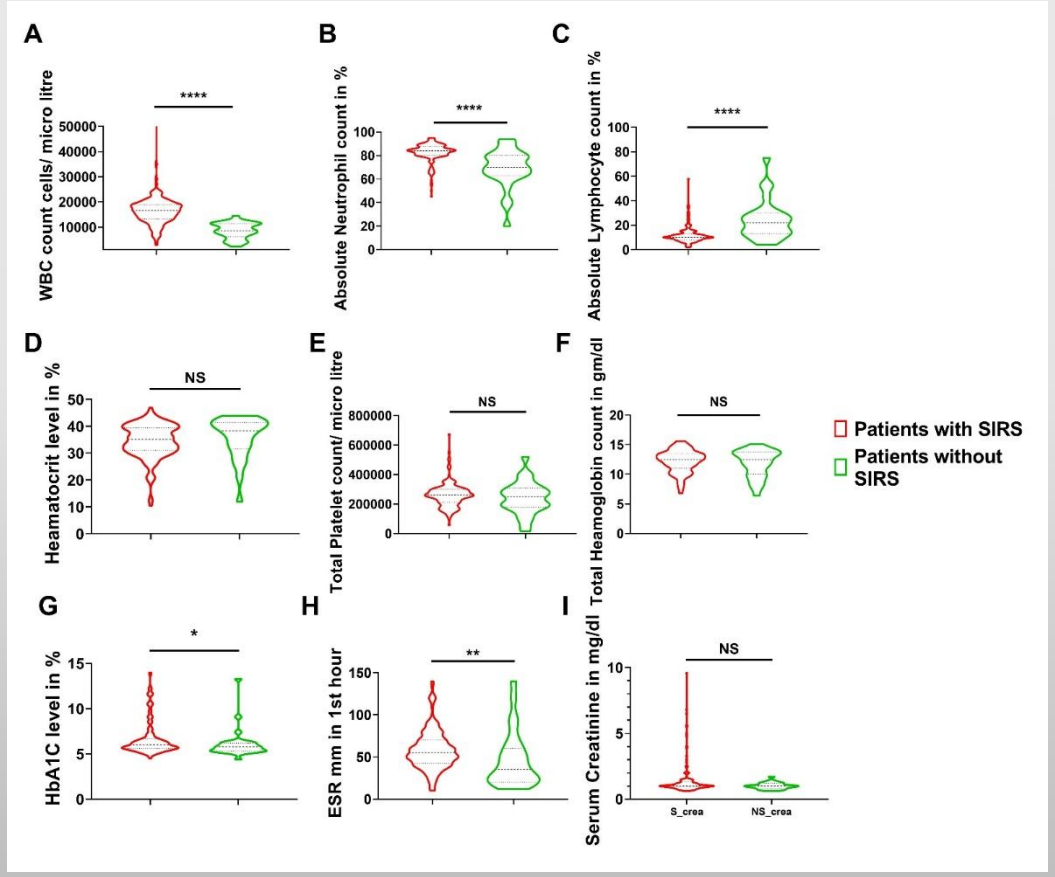
- **15 ml of blood only from culture positive for Gram-negative bacteria for PBMC, plasma and serum**
- **Latex test to confirm the *B. pseudomallei***

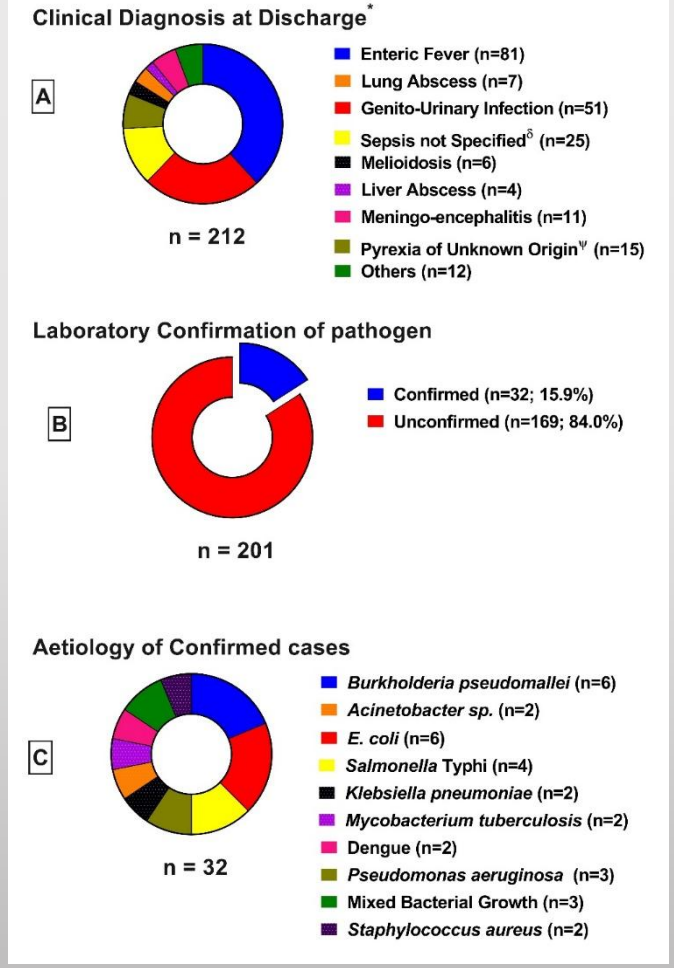


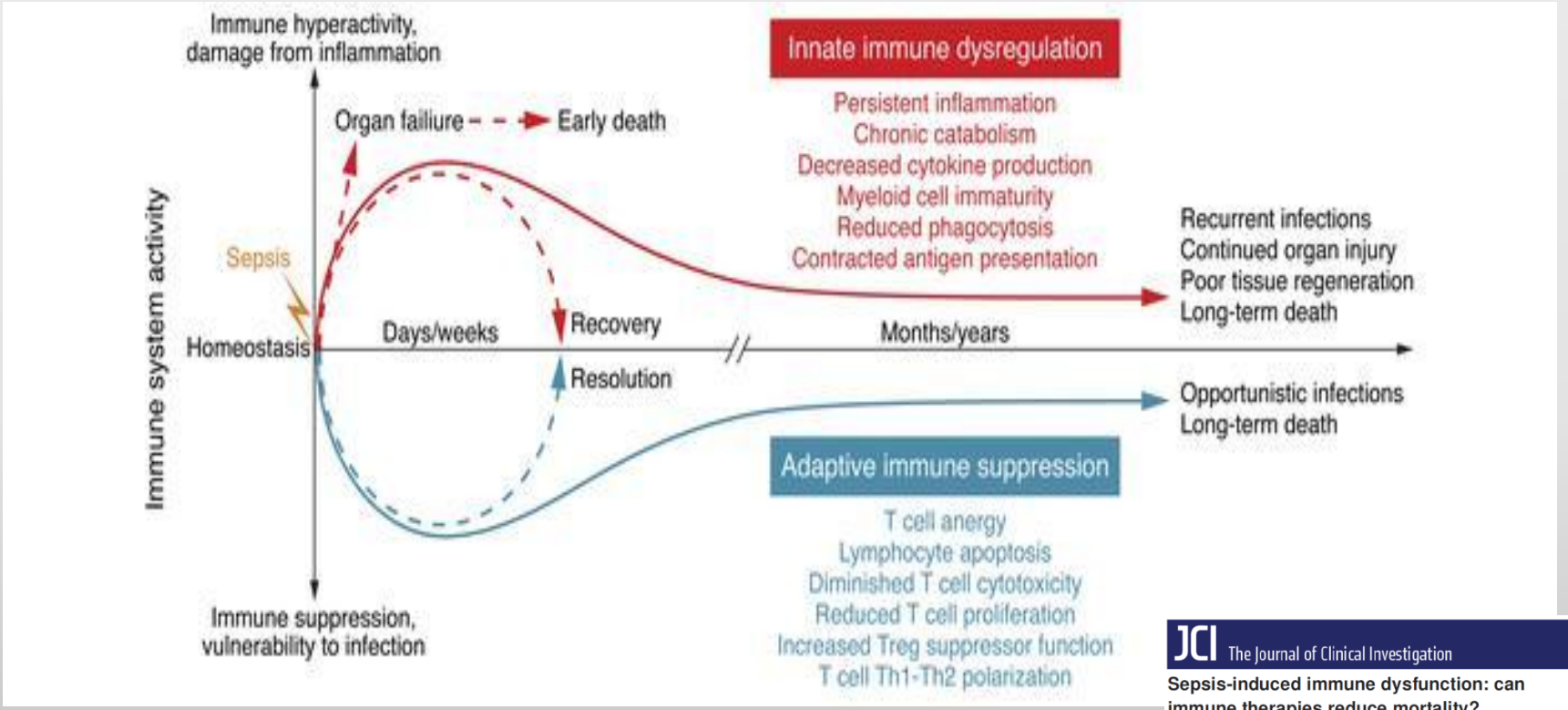
Variables	Number	Percentage
Sex		
Male	119	57
Female	91	43
Age (Median, IQR)	35 (23-55)	
Occupation		
Farmer	28	13
Student	55	26
Housewife	69	33
Worker/ day labourer	41	20
Business	12	6
Others	05	2
Average distance to hospital from home (KM)	124 (1-450)	
Lag period between symptom and hospitalization (days)	10 (6-15)	



- 86.5% (174) patients admitted with SIRS and 13.5% (27) were without SIRS
- A quarter (51; 24.2%) of the patients found to be diabetic
- 74 (35.2%) patients were exposed to Hepatitis B virus and prevalence of Hep B among febrile patients was 6.6% (14)
- ESBL producing *E. coli* and *Klebsiella* infection was high. MDR typhoid was also high in Dhaka
- The total mortality was 15.7% (33).


A
WBC count cells/ micro litre
50000
40000
30000
20000
10000
****
B
Absolute Neutrophil count in %
100
80
60
40
20
0
****
C
Absolute Lymphocyte count in %
100
80
60
40
20
0
****
D
Heamatocrit level in %
50
40
30
20
10
0
NS
E
Total Platelet count/ micro litre
800000
600000
400000
200000
0
NS
F
Total Heamoglobin count in gm/dl
20
15
10
5
0
NS
Patients with SIRS
Patients without SIRS
G
HbA1C level in %
15
10
5
0
*
H
ESR mm in 1st hour
150
100
50
0
**
I
Serum Creatinine in mg/dl
10
5
0
NS
S_crea
NS_crea


Clinical Diagnosis at Discharge*
A
n = 212
Enteric Fever (n=81)
Lung Abscess (n=7)
Genito-Urinary Infection (n=51)
Sepsis not Specified$^{\delta}$ (n=25)
Melioidosis (n=6)
Liver Abscess (n=4)
Meningo-encephalitis (n=11)
Pyrexia of Unknown Origin$^{\Psi}$ (n=15)
Others (n=12)
Laboratory Confirmation of pathogen
B
n = 201
Confirmed (n=32; 15.9%)
Unconfirmed (n=169; 84.0%)
Aetiology of Confirmed cases
C
n = 32
Burkholderia pseudomallei (n=6)
Acinetobacter sp. (n=2)
E. coli (n=6)
Salmonella Typhi (n=4)
Klebsiella pneumoniae (n=2)
Mycobacterium tuberculosis (n=2)
Dengue (n=2)
Pseudomonas aeruginosa (n=3)
Mixed Bacterial Growth (n=3)
Staphylococcus aureus (n=2)



JCI The Journal of Clinical Investigation

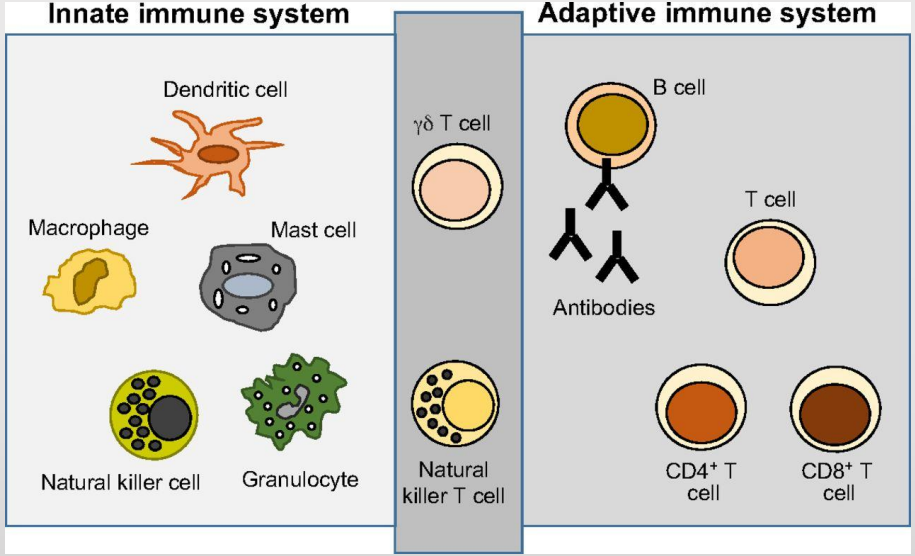
Sepsis-induced immune dysfunction: can immune therapies reduce mortality?

Matthew J. Delano, Peter A. Ward

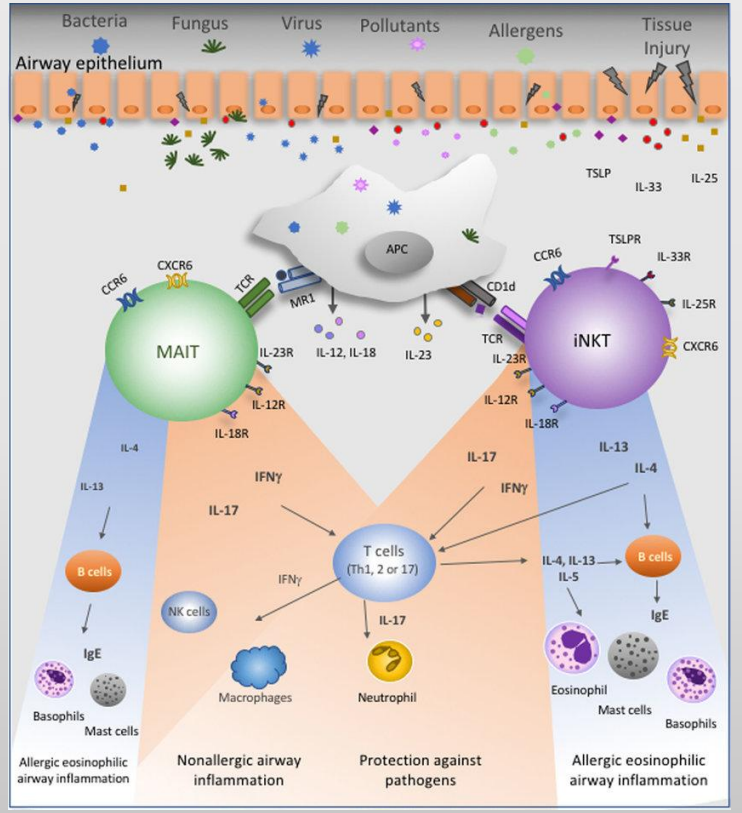
J Clin Invest. 2016;126(1):23-31. https://doi.org/10.1172/JCI82224.

Review

Sepsis-induced immune dysfunction



Ref: Disease Models & Mechanisms 2015 8: 337-350;
doi: 10.1242/dmm.018036



Front. Immunol. 9:1766. doi: 10.3389/fimmu.2018.01766

Brief Reviews

The Journal of Immunology

Sepsis-Induced T Cell Immunoparalysis: The Ins and Outs of Impaired T Cell Immunity

Isaac J. Jensen,* Frances V. Sjaastad,† Thomas S. Griffith,†,‡,§,¶ and Vladimir P. Badovinac*,‖,#

CVI
Journals.ASM.org

Association of γδ T Cells with Disease Severity and Mortality in Septic Patients

Juan C. Andreu-Ballester,[a] Constantino Tormo-Calandín,[b] Carlos Garcia-Ballesteros,[c] J. Pérez-Griera,[d] Victoria Amigó,[e] Amadeo Almela-Quilis,[f] Juan Ruiz del Castillo,[g] Carlos Peñarroja-Otero,[h] Ferran Ballester[i]

Andaluz-Ojeda *et al. Critical Care* 2011, **15**:R243
http://ccforum.com/content/15/5/R243

CRITICAL CARE

RESEARCH Open Access

Early natural killer cell counts in blood predict mortality in severe sepsis

David Andaluz-Ojeda[1], Verónica Iglesias[2], Felipe Bobillo[1], Raquel Almansa[2], Lucía Rico[2], Francisco Gandía[1], Ana Ma Loma[2], Concepción Nieto[2], Rosa Diego[2], Epifanio Ramos[2], Mercedes Nocito[2], Salvador Resino[4], Jose M Eiros[2], Eduardo Tamayo[3], Raul Ortiz de Lejarazu[2] and Jesús F Bermejo-Martin[2*]

OPEN

Experimental & Molecular Medicine (2017) 49, e382; doi:10.1038/emm.2017.146
Official journal of the Korean Society for Biochemistry and Molecular Biology
www.nature.com/emm

ORIGINAL ARTICLE

Impaired polyfunctionality of CD8+ T cells in severe sepsis patients with human cytomegalovirus reactivation

Young Joon Choi[1,5], Sun Bean Kim[2,5], Jong Hoon Kim[1], Su-Hyung Park[3], Moo Suk Park[4], June Myung Kim[2], Sang Hoon Han[2] and Eui-Cheol Shin[1]

HHS Public Access
Author manuscript
Crit Rev Immunol. Author manuscript; available in PMC 2017 February 17.

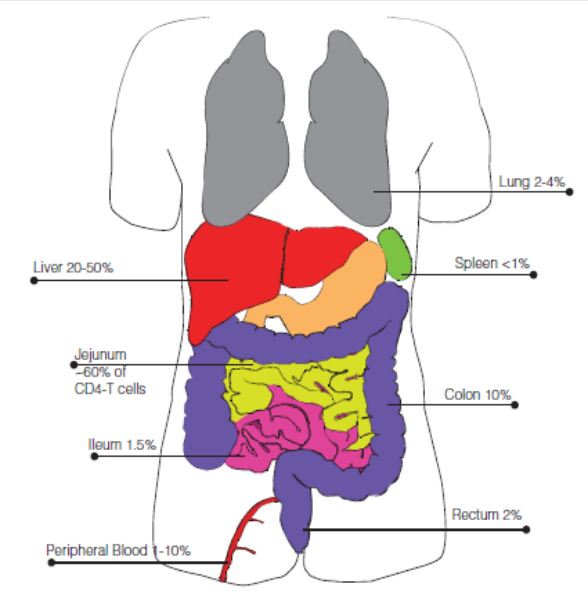
Published in final edited form as:
Crit Rev Immunol. 2016 ; 36(1): 57–74. doi:10.1615/CritRevImmunol.2016017098.

Clinical and Experimental Sepsis Impairs CD8 T-Cell-Mediated Immunity

Derek B. Danahy[a,b], **Robert K. Strother**[a], **Vladimir P. Badovinac**[a,b], and **Thomas S. Griffith**[c,d,e,f,*]

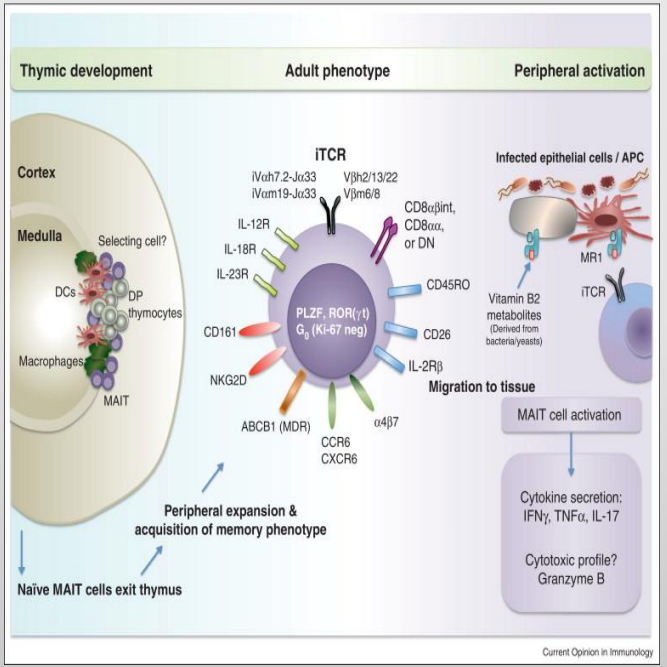
Mucosal Associated Invariant T cell

- MAIT cells are a subset of innate-like T cells with both innate and adaptive immune properties.
- These cells are abundant in the gut, liver and blood, comprising 1-10% of peripheral blood T lymphocytes.
- MAIT cells express a conserved invariant T cell receptor (TCR) α-chain (Vα7.2-Jα33) in humans, and are defined by their restriction to the non-classical MHC class I-related (MR1) molecule.

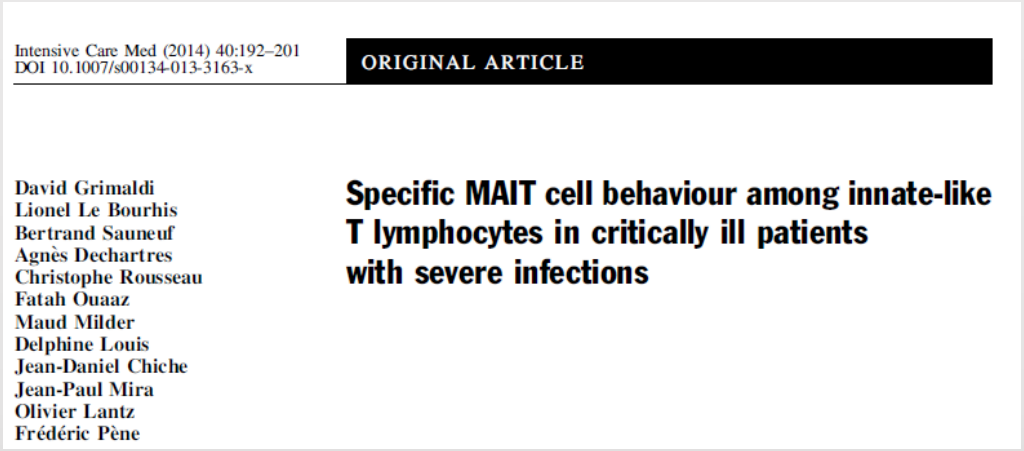


Ref: Kurioka A et al. MAIT cells: new guardians of the liver. Clin Trans Immunology 2016;5(8):e98

- Characterized by expression of Vα7.2 and CD161 ++ on their surface.
- Majority are CD8+ and CD4-CD8- (DN) population, with CD4+ in rare cases.
- Microbes (Gram +/- bacteria, Yeasts) that possess Riboflavin precursors are able to stimulate MAIT cells. Also activated in response to viruses and fungi.
- Upon activation produce CKs:
  - IL22, IL17, IFNγ, TNFα
  - Granzyme B, perforin, Granulysin.



Ref: Bourhis LL et al, Curr Op Imm 2013; 25(2): 174-80



Intensive Care Med (2014) 40:192–201
DOI 10.1007/s00134-013-3163-x

ORIGINAL ARTICLE

David Grimaldi
Lionel Le Bourhis
Bertrand Sauneuf
Agnès Dechartres
Christophe Rousseau
Fatah Ouaaz
Maud Milder
Delphine Louis
Jean-Daniel Chiche
Jean-Paul Mira
Olivier Lantz
Frédéric Pène

Specific MAIT cell behaviour among innate-like T lymphocytes in critically ill patients with severe infections

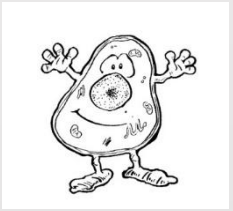
- Patients with severe bacterial infections displayed an early decrease in MAIT cell count as compared to control healthy subjects, but also to non-infected critically ill patients
- In contrast NKT and γδ T cell counts did not differ between patients groups

Aims

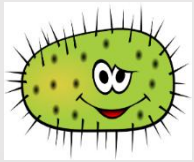
- To track the phenotype, activation and function of MAIT cells in patients infected with acute Gram-negative bacteria from Bangladesh and Thailand (*E. coli*, Salmonella *Typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*)

- To compare these responses with healthy endemic control and critically ill non-infectious patients from the same setting

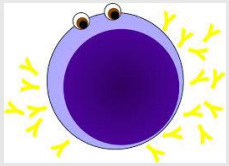
FACS experiment design



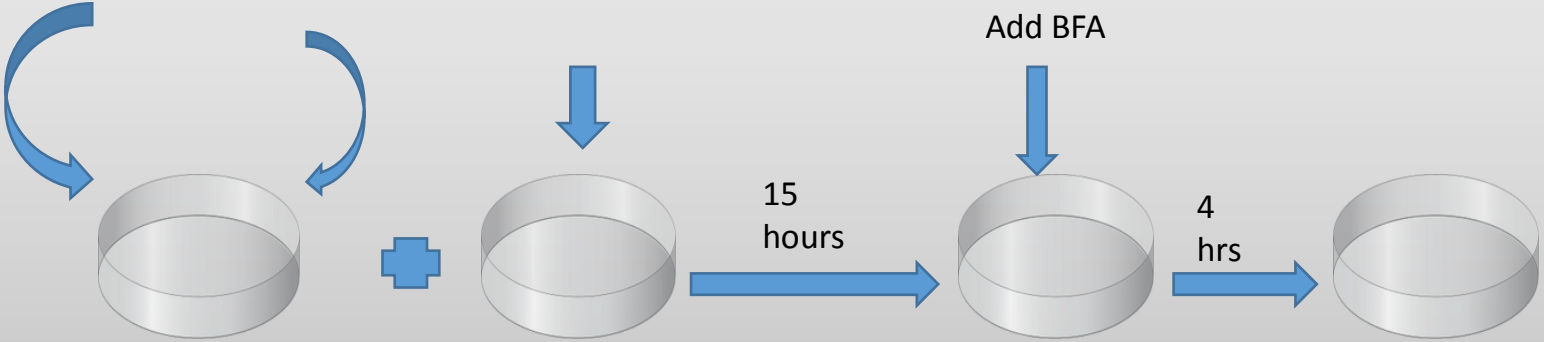
THP1
80,000/well)



E.Coli/
Salmonella/
Klebsiella/
Pseudomonas



PBMC
1x10^6

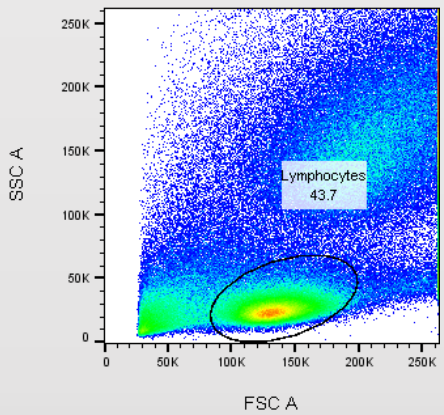


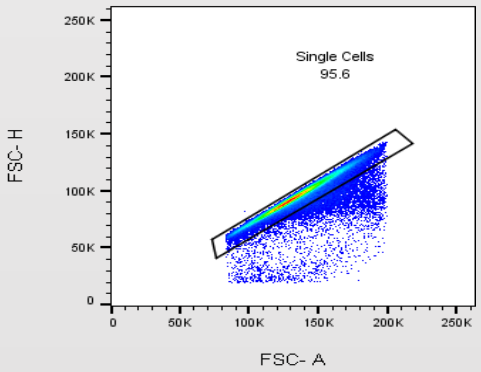
Co-Culture of THP1
and Bacteria

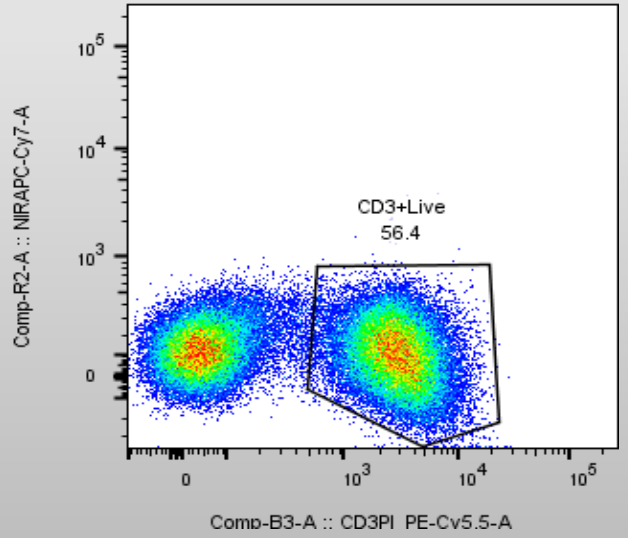
Adding cells

Incubation at
37^0 C, 5% CO2

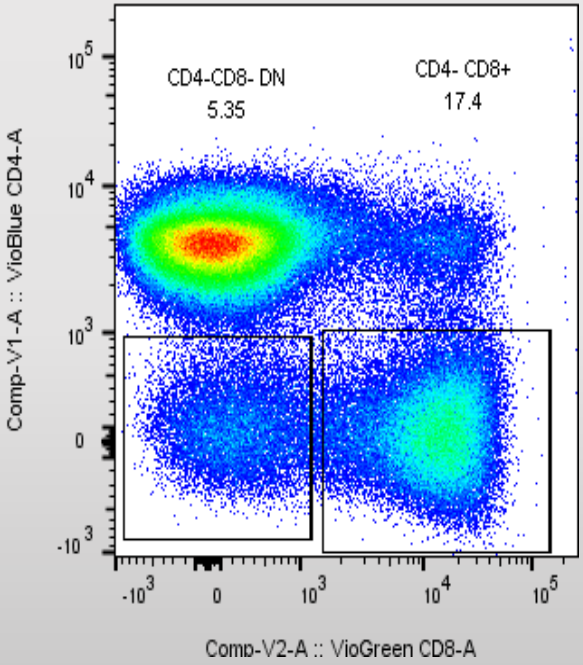
ECS and ICS
staining

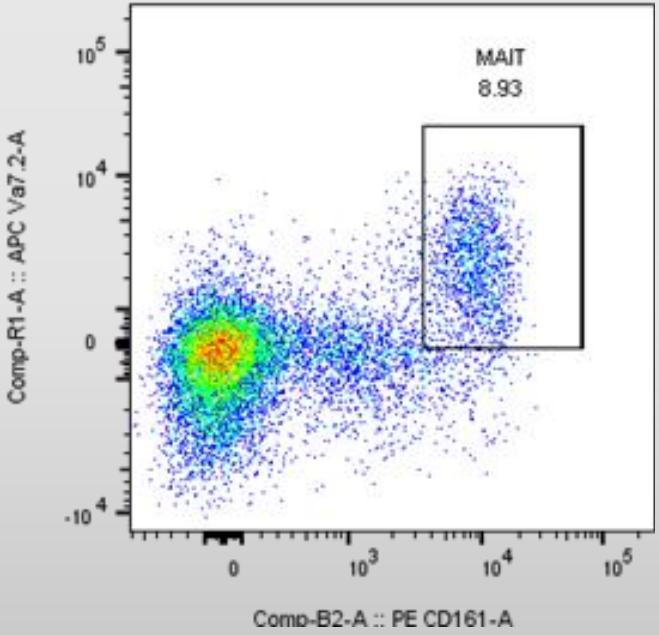


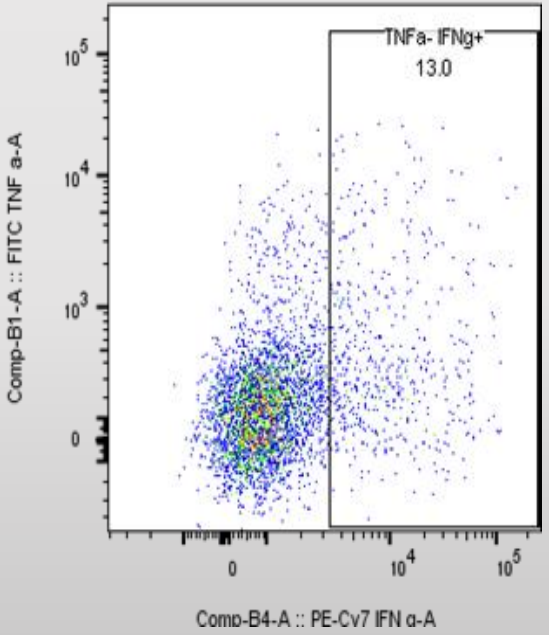




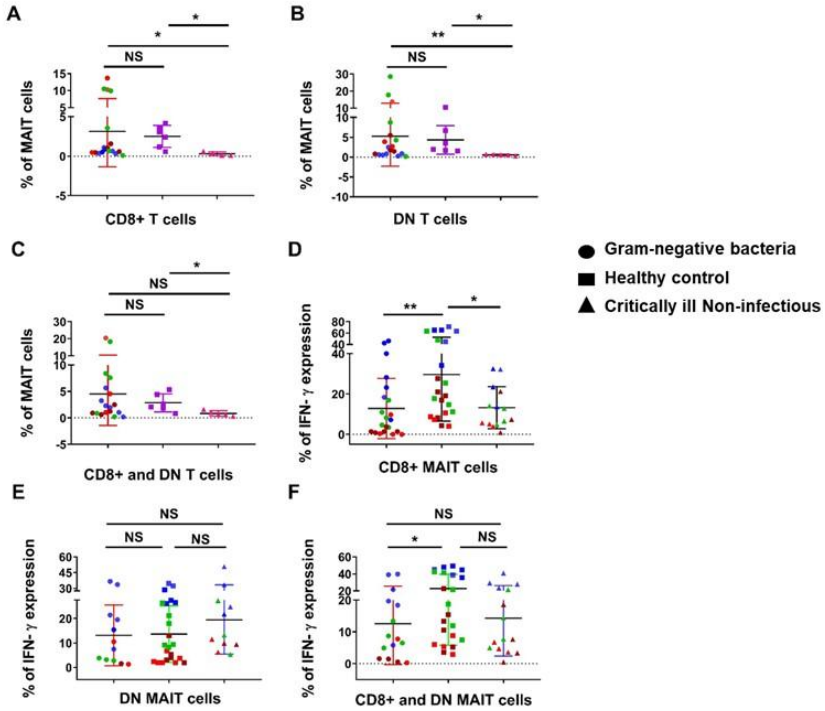
Gating Strategy


CD4-CD8- DN
5.35
CD4- CD8+
17.4
Comp-V1-A :: VioBlue CD4-A
Comp-V2-A :: VioGreen CD8-A

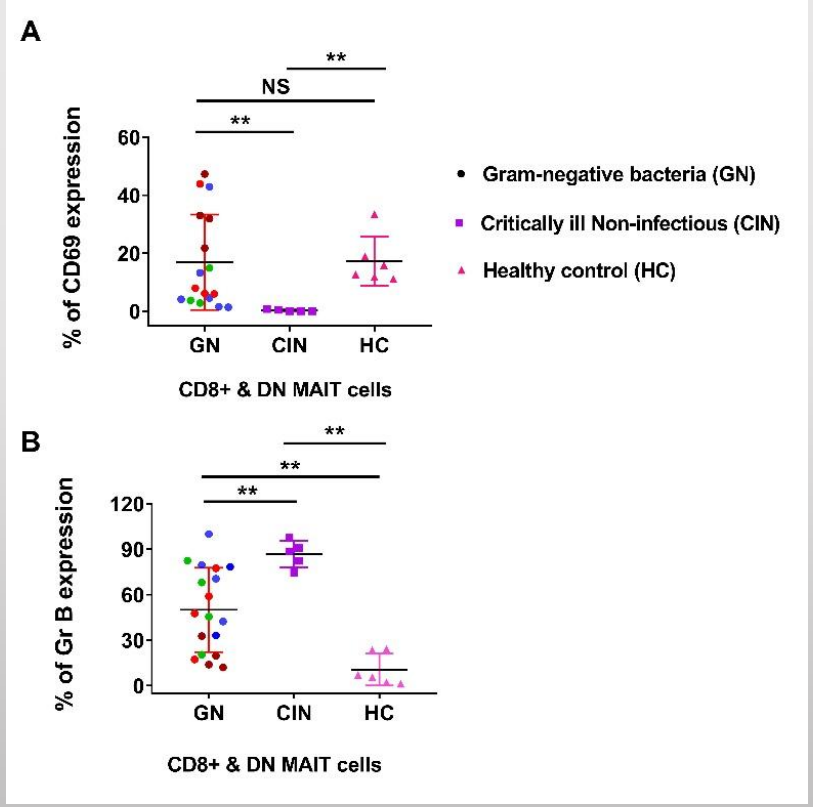

MAIT
8.93
Comp-R1-A :: APC Va7.2-A
Comp-B2-A :: PE CD161-A


TNFa- IFNg+
13.0
Comp-B1-A :: FITC TNF a-A
Comp-B4-A :: PE-Cy7 IFN g-A

IFN-γ expression by MAIT cells is reduced in patients infected with different Gram-negative bacteria compared to healthy controls



The cytotoxic effector molecule GzmB is significantly higher in Gram-negative patients compared to healthy control, without any difference in activation



Limitations and Recommendations

- Sample size. We have compiled all the Gram-negative samples. Response to individual bacteria could not be explored
- Could not evaluate the role of MAIT cell on survival due to small number of samples
- Larger longitudinal clinical studies in Gram-negative sepsis patients is required
- Clinical management is not standardized. No guideline available based on local antibiogram

Conclusion

- Disruption of CD8 T cells and its various subsets (particularly MAIT cells) functionality could be an important consequence of Gram-negative sepsis

- It is evidenced by low IFN-γ release and higher cytotoxic molecule GzmB expression

Acknowledgments

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MORU, Bangkok

Manutsanun Sumonwiriya (Ka)
Kemajittra Jenjaroen (Ma)
Dr Panjaporn Chaichana (Por)
Prof Direk Limmathurotsakul
Aj Vanaporn Wuthiekanun
Dr Narisara Chantratita
Prof. Sir Nicholas White
Prof Nicholas Day

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