LETTER TO THE EDITOR

Temporal changes in erythroid progenitors in critically ill patients: a prospective cohort study

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Online Data Supplement

Supplementary Table S1

Table S1A - Detailed inclusion and exclusion criteria;

Inclusion criteria

- ICU patients (≥16 years old), both with capacity and without capacity (for medically induced reasons such as therapeutic sedation), who have required at least 72 hours of adult ICU care

Exclusion criteria

- Active haematological malignancy
- Chemotherapy or myelosuppressive treatment within last 30 days
- Documented or suspected HIV infection
- Received massive transfusion during index admission defined as 'replacement of >1 blood volume in 24 hours or >50% of blood volume in 4 hours'.
- Palliative care intent
- Participants who are lacking capacity for non-medically induced reasons e.g. dementia
- Death is imminent or likely during this admission
- Patients residing outside a reasonable geographic follow-up area (defined as within 30 miles of the John Radcliffe Hospital, Oxford, UK)

S1B - Characteristics of study cohort with paired samples (n=17)

Characteristic	Value
Age (years)	58 (50 – 63)
Sex, n (%)	
Male	8 (47.0)
Female	9 (53.0)
BMI ($kg.m^{-2}$)	27.0 (23.5 – 35.9)
Functional Comorbidity Index, n (%)	
0	5 (29.5)
1	4 (23.5)
2	4 (23.5)
>3	4 (23.5)
APACHEII score	21.8 (6.1)
Admission diagnoses, n (%)	
Elective operation	0(0.0)
Emergency operation	9 (53.0)
Medical	8 (47.0)
No. with septic shock, n (%)	8 (47.0)
Organ support requirements, n (%)	
Advanced respiratory support	16 (94.1)
Advanced cardiovascular support	9 (53.0)
Advanced renal support	13 (76.4)
PaO_2 / FiO_2 ratio (mmHg)	160 (128 – 248)

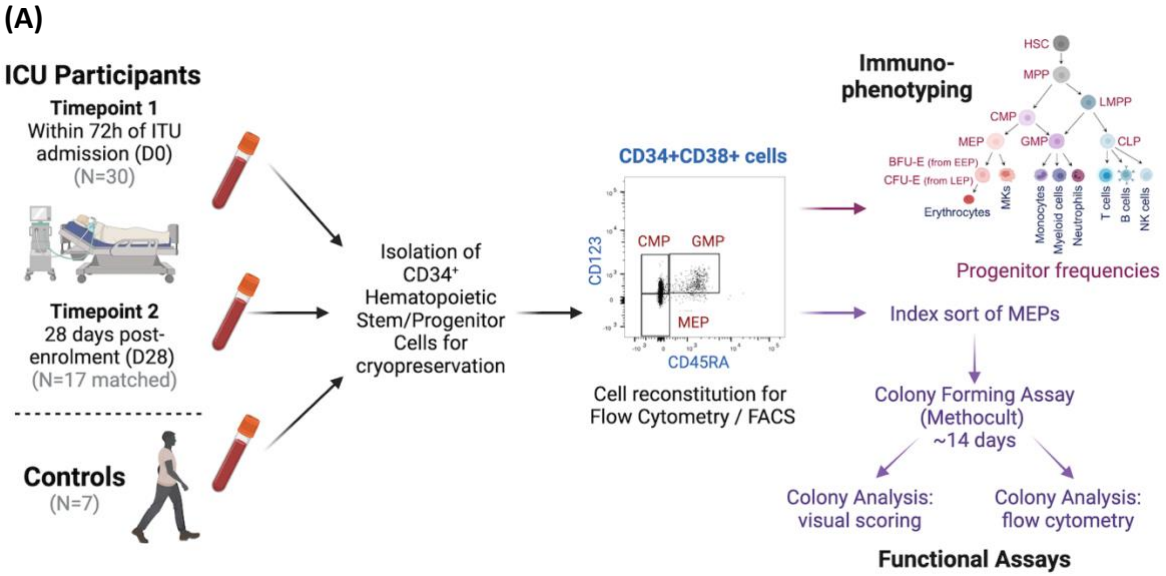
ICU length of stay, days	9 (6 – 17)

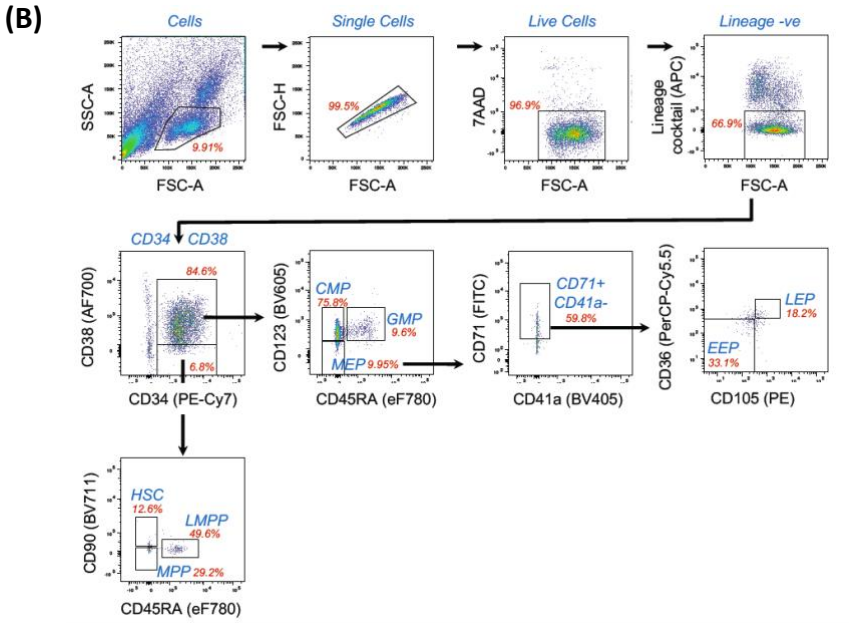
APACHEII, Acute Physiology and Chronic Health Evaluation; BMI, Body mass index; CRP, C-reactive protein; EPO, erythropoietin; ICU, Intensive care unit; IQR, interquartile range; SD, standard deviation

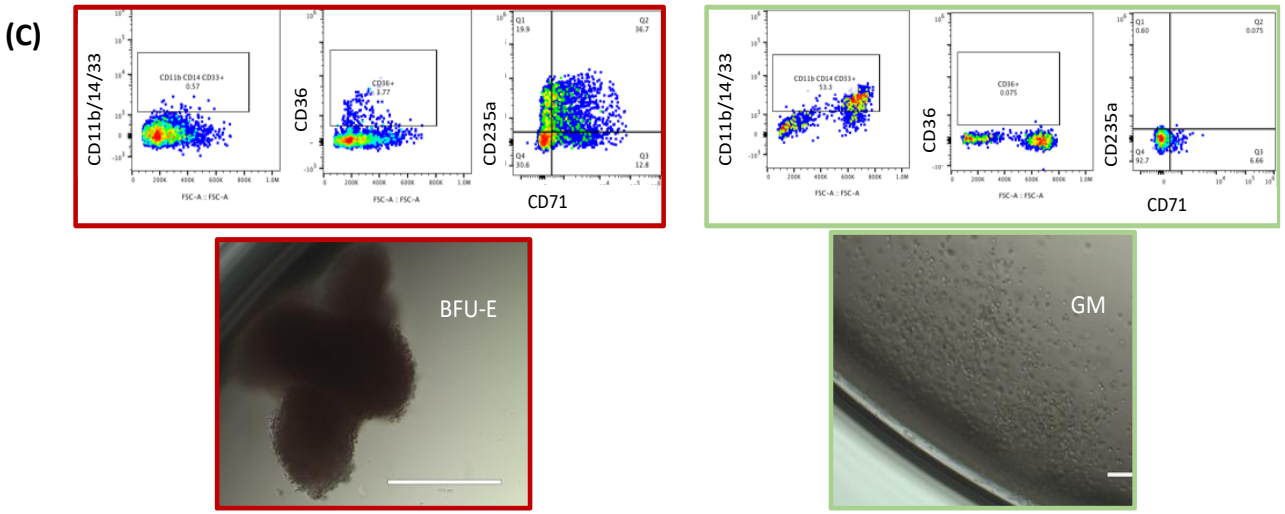
Supplementary Table S2: Details of antibodies used

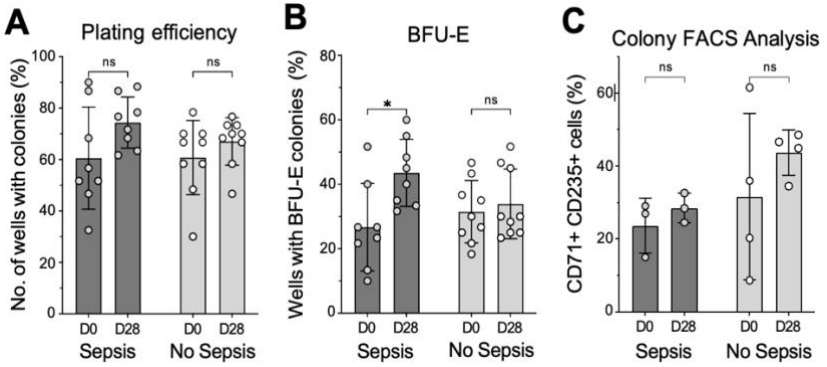
Anti-human antibodies used for analysis and sorting			
Antibody	Clone	Fluorochrome	Supplier
CD34	4H11	PE-Cy7	eBioscience
CD36	CB38	PerCP-Cy5.5	BD Biosciences
CD71	OKT9	FITC	eBioscience
Lineage Cocktail (CD2, CD3, CD14, CD16, CD19, CD56, CC235)	n/a	APC	eBioscience
CD105	43A3	PE	Biolegend
CD38	HIT2	AF700	eBioscience
CD123	7G3	BV605	BD Biosciences
CD235a	HIR2	PerCp-Cy5	Biolegend
CD45RA	Hl100	APC-eFluor780	eBioscience
CD90	5E10	BV711	BD Biosciences
CD41a	HIP8	AlexaFluor405	eBioscience
Viability dye	na	7AAD	Life Technologies
Antibodies used for FACS analysis of colonies			
CD235	HIR2	PE	Life Technologies
CD71	CY1G4	PE-Cy7	Biolegend
CD36	CB38	APC	BD Bioscience
CD11b	ICRF44	FITC	eBioscience
CD14	6ID3	FITC	Biolegend
CD33	P67.6	FITC	Biolegend
Viability dye	na	Hoechst	Invitrogen

Supplementary Figure S1









Supplementary Figure

S1A Overview of the experimental strategy.

$CD34^+$ haematopoietic and stem progenitor cells (HSPCs) were extracted from controls (n=7) or study participants within 72 hours of admission to an intensive care unit (ICU) (D0 baseline samples) or 28 days later (D28) and cryopreserved. We had seven controls in total – all of whom were healthy volunteers (4 males, 3 females, age range 24-48), and were recruited from our research facility. Four of these volunteers (all male) provided blood samples every 4-6 weeks in parallel with batch analysis of study participants' samples. Peripheral blood was also collected from the remaining three volunteers (all female) at one timepoint. Cells were immuno-stained with a 12-colour fluorochrome panel. For frequency analysis, proportions of different cellular subsets were determined from flow cytometry plots with gates set using Fluorescence-minus-one controls and single stains. For functional analysis, Megakaryocyte-Erythroid Progenitors (MEPs) were index sorted into 96 well plates containing Methocult; colonies were allowed to grow for 14 days before being imaged and selected for FACS analysis to determine colony lineage. *Abbreviations*: HSC = Haematopoietic Stem Cell; MPP = Multipotent Progenitor; CMP = Common Myeloid Progenitor; LMPP = Lymphoid-primed Multipotent Progenitor; MEP = Megakaryocyte-Erythroid Progenitor; GMP = Granulocyte-Monocyte Progenitor ; CLP = Common Lymphoid Progenitor; BFU-E = Blast-forming Unit - Erythroid ; EEP = Early Erythroid Progenitor; CFU-E = Colony-forming Unit - Erythroid; LEP = Late Erythroid Progenitor; MKs = Megakaryocytes; NK = Natural Killer.

S1B FACS gating/sorting strategy.

$CD34^+$ HSPCs were immuno-stained with a 12 colour fluorochrome panel and gates were set using FMOs and single stains.

S1C Colony analysis by FACS.

Representative examples of a BFU-E and GM colony grown for 14 days in Methocult from single cell sorted MEPs (defined by flow cytometry as Lin^- $CD34^+$ $CD38^+$ $CD45RA^-$ $CD123^-$) (A) Flow cytometric analysis shows expression of the erythroid markers (CD71, CD36 and CD235) and myeloid markers (CD11b/CD14/ and CD33) (B) images of a BFU-E and GM colony.

S1D Patients admitted with sepsis showed a significant increase in the number of BFU-Es at D28 compared to baseline and this was not observed in non-sepsis patients. (A) Plating efficiency of single index sorted MEPs from all participants showing the effect of sepsis (n=8) vs non-sepsis (n=9). (B) The number of Burst Forming Units-Erythroid (BFU-Es) grown from MEPs 14 days after single-cell sorting into methylcellulose from pair-matched participants admitted with either sepsis (n=8) or non-sepsis (n=9). (C) Flow cytometric analysis of BFU-E colonies at day 14 showing the expression levels of the erythroid markers $CD71^+$ $CD235^+$ (data available from n=3 with sepsis and n=4 non-sepsis). Two-way repeated measures ANOVA; overall effect of timepoint in (A) $p=0.024$ and in (B) $p=0.035$; *$p<0.05$ Sidak's multiple comparison test.