SUPPLEMENTARY MATERIAL

Appendix 1.
The detailed search strategy is showed as follow:

PUBMED=317

1. ((((erythrocyte*) OR red cell*) OR red blood cell*) OR RBC*) OR blood
2. ((retransfus*) OR transfuse*) OR infuse*
3. ((((((((*Time Factors) OR Blood Preservation) OR age*) OR aging) OR fresh*) OR old*) OR new*) OR young*) OR store*) OR storage) OR storing) OR preserv*
4. (((((Critical Illness) OR Critical Care) OR Intensive Care) OR Intensive Care Units) OR Critically ill) OR Critical*) OR Intensive*
5. #1 AND #2 AND #3 AND #4
6. ((time factors[MeSH Terms]) AND (((erythrocyte transfusion[MeSH Terms]) OR erythrocytes[MeSH Terms]) OR blood component transfusion[MeSH Terms]) OR blood transfusion[MeSH Terms])) AND (((Intensive Care Units[MeSH Terms]) OR Intensive Care[MeSH Terms]) OR Critical Illness[MeSH Terms]) OR Critical Care[MeSH Terms])
7. #5 OR #6
8. #7 Filters: Clinical Trial; Humans

OVID=794

1. (erythrocyte* or red cell* or red blood cell* or RBC* or blood).mp. [mp=title, abstract, full text, caption text]
2. (retransfus* or transfuse* or infuse*).mp. [mp=title, abstract, full text, caption text]
3. (Blood Preservation or age* or aging or fresh* or old* or new* or young* or store* or storage or storing or preserv*).mp. [mp=title, abstract, full text, caption text]
4. (Critical Illness or Critical Care or Intensive Care or Intensive Care Units or
Critically ill or Critical* or Intensive*).mp. [mp=title, abstract, full text, caption text]

5. #1 AND #2 AND #3 AND #4

6. #5 AND "Article" [Publication Type] AND yr="1860-2017".

**Web of Science=2717**

TS=(erythrocyte* or red cell* or red blood cell* or RBC* or blood) AND TS=(retransfus* or transfuse* or infuse*) AND TS=(Blood Preservation or age* or aging or fresh* or old* or new* or young* or store* or storage or storing or preserv*) AND TS=(Critical Illness or Critical Care or Intensive Care or Intensive Care Units or Critically ill or Critical* or Intensive*)

**Cochrane Library=30**

1. MeSH descriptor: [Erythrocyte Transfusion] explode all trees
2. MeSH descriptor: [Erythrocytes] explode all trees
3. MeSH descriptor: [Blood Component Transfusion] explode all trees
4. MeSH descriptor: [Blood Transfusion] explode all trees
5. #1 or #2 or #3 or #4
6. MeSH descriptor: [Time Factors] explode all trees
7. MeSH descriptor: [Intensive Care Units] explode all trees
8. MeSH descriptor: [Critical Care] explode all trees
9. MeSH descriptor: [Critical Illness] explode all trees
10. #7 or #8 or #9
11. #5 and #6 and #10
Appendix 2. The reasons for exclusion of the 23 ineligible studies are listed as follow:

<table>
<thead>
<tr>
<th>Trials</th>
<th>Reasons for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacroix/2015[s1]</td>
<td>Duplicate publication (1 trial)</td>
</tr>
<tr>
<td>Walsh/2004[s2]; Damiani/2015[s3]</td>
<td>without any endpoints (2 trials)</td>
</tr>
<tr>
<td>Yamal/2015[s4]; Cywinski/2013[s5]</td>
<td>Non-randomized trials (2 trials)</td>
</tr>
<tr>
<td>Leal-Noval/2003[s6]</td>
<td>Prospective cohort study (1 trial)</td>
</tr>
<tr>
<td>Cartotto/2014[s7]</td>
<td>Retrospective study (1 trial)</td>
</tr>
<tr>
<td>Leal-Noval/2008[s8]; Kaukonen/2013[s9]; Pettilä/2011[s10]</td>
<td>Observational studies (3 trial)</td>
</tr>
<tr>
<td>Hebert/2005[s11]</td>
<td>The subjects in this study, which enrolled 42 patients undergoing cardiovascular surgery and 15 critically ill patients, are not exactly critically ill patients.</td>
</tr>
<tr>
<td>Heddle/2012[s12]</td>
<td>Although this trial was conducted in acute care hospital, the subjects was enrolled from emergency department, clinic or other department, but not from ICU.</td>
</tr>
<tr>
<td>Weiskopf/2006[s13]; van de Watering/2006[s14]; Bennett-Guerrero/2009[s15]; Hod/2011[s16]; Roberson/2012[s17]; Berra/2014[s18]; Neuman/2015[19]; Steiner/2015[s20]; Risbano/2015[s21]; Bao/2017[s22]; Spadaro/2017[s23]</td>
<td>The subjects are not critically ill patients admitted to ICU (11 trials)</td>
</tr>
</tbody>
</table>
SUPPLEMENTARY REFERENCE


17(5):R222.
s11. Hébert PC, Chin-Yee I, Fergusson D, Blajchman M, Martineau R,
Clinch J, et al. A pilot trial evaluating the clinical effects of prolonged 
s12. Heddle NM, Cook RJ, Arnold DM, Crowther MA, Warkentin TE, Webert 
randomized controlled pilot feasibility trial. Transfusion.
Fresh blood and aged stored blood are equally efficacious in immediately 
reversing anemia-induced brain oxygenation deficits in humans. 
Effects of storage time of red blood cell transfusions on the prognosis of 
s15. Bennett-Guerrero E, Stafford-Smith M, Waweru PM, Bredehoeft SJ,
Campbell ML, Haley NR, et al. A prospective, double-blind, randomized 
clinical feasibility trial of controlling the storage age of red blood cells for 
s16. Hod EA, Brittenham GM, Billote GB, Francis RO, Ginzburg YZ,
Hendrickson JE, et al. Transfusion of human volunteers with older, stored 
red blood cells produces extravascular hemolysis and circulating 
s17. Roberson RS, Lockhart E, Shapiro NI, Bandarenko N, McMahon TJ,
AS-3 red blood cells on tissue oxygenation and the microcirculation in 


SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Forest plot of meta-analysis for the duration of intensive care unit stay. Mean and SD on the log scale and the unit is log hours. RBC red blood cell; SD standard deviation; IV inverse variance.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Fresher RBCs</th>
<th>Older RBCs</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>Aubron/2012</td>
<td>5.39</td>
<td>0.61</td>
<td>25</td>
<td>4.54</td>
</tr>
<tr>
<td>Cooper/2017</td>
<td>4.12</td>
<td>0.99</td>
<td>2457</td>
<td>4.11</td>
</tr>
<tr>
<td>Lacroix/2015</td>
<td>5.46</td>
<td>0.84</td>
<td>1206</td>
<td>5.48</td>
</tr>
<tr>
<td>Schreiber/2015</td>
<td>4.57</td>
<td>0.9</td>
<td>82</td>
<td>4.28</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>3770</td>
<td></td>
<td>3780</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 16.21, df = 3 (P = 0.001); I² = 81%
Test for overall effect: Z = 0.47 (P = 0.64)
Supplementary Figure 2. Forest plot of meta-analysis for the duration of hospital stay. Mean and SD on the log scale and the unit is log hours. RBC red blood cell; SD standard deviation; IV inverse variance.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Fresher RBCs</th>
<th>Older RBCs</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>Aubron/2012</td>
<td>5.92</td>
<td>0.78</td>
<td>25</td>
<td>5.75</td>
</tr>
<tr>
<td>Cooper/2017</td>
<td>5.49</td>
<td>0.85</td>
<td>2457</td>
<td>5.49</td>
</tr>
<tr>
<td>Lacroix/2015</td>
<td>6.3</td>
<td>0.92</td>
<td>1206</td>
<td>6.28</td>
</tr>
<tr>
<td>Schreiber/2015</td>
<td>5.54</td>
<td>0.64</td>
<td>82</td>
<td>5.69</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>3770</td>
<td></td>
<td>3780</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: \( \chi^2 = 3.24, \, df = 3 \, (P = 0.36); \, I^2 = 7\%

Test for overall effect: \( Z = 0.05 \, (P = 0.96) \)
Supplementary Figure 3. Sensitivity analysis for assessing the robustness of pooled OR for short-term mortality. The overall effect of storage age of transfused RBCs on short-term mortality was unchanged after sequentially removing one study at a time.
Supplementary table 1. The conventional meta-analysis and TSA using random-effects and fixed-effects for all endpoints.

<table>
<thead>
<tr>
<th></th>
<th>Conventional meta-analysis</th>
<th>TSA, with α of 5%, power of 80%, relative risk reduction of 15%, two-tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Random-effects</td>
<td>Fixed-effects</td>
</tr>
<tr>
<td>OR(95%CI)</td>
<td>P</td>
<td>I²</td>
</tr>
<tr>
<td>All trials</td>
<td>1.04(0.96 - 1.13)</td>
<td>0.31</td>
</tr>
<tr>
<td>Multi-center trials</td>
<td>1.04(0.96 - 1.13)</td>
<td>0.29</td>
</tr>
<tr>
<td>Single-center trials</td>
<td>1.16(0.28 - 4.71)</td>
<td>0.83</td>
</tr>
<tr>
<td>Trials with Low risk of bias</td>
<td>1.04(0.94 - 1.16)</td>
<td>0.44</td>
</tr>
<tr>
<td>Secondary endpoints</td>
<td>Mean difference (95%CI) on the log scale</td>
<td></td>
</tr>
<tr>
<td>Duration of ICU stay</td>
<td>0.10(-0.03 to 0.01)</td>
<td>0.16</td>
</tr>
<tr>
<td>Duration of hospital stay</td>
<td>0.00(-0.00 to 0.00)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The older RBCs group was taken as a reference. ICU intensive care unit; OR odds ratio; CI confidence interval; TSA trial sequential analysis.