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Why have meta-analyses of randomized controlled trials of the association between non-white-blood-cell-reduced allogeneic blood transfusion and postoperative infection produced discordant results?

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Vox Sanguinis

Intention-to-treat analyses of randomized controlled trials (RCTs) of the association between non-white-blood-cell (WBC)-reduced allogeneic blood transfusion (ABT) and postoperative infection were reported as the reason why meta-analyses of RCTs of this association have produced discordant results. We examined three possible reasons for disagreements between meta-analyses: (i) sources of medical heterogeneity and integration of RCTs despite extreme heterogeneity; (ii) reliance on as-treated (vs. intention-to-treat) comparisons; and (iii) inclusion (or not) of the three most recent RCTs. When nine RCTs reported up to 2002 were combined despite extreme heterogeneity, both intention-to-treat and as-treated comparisons found an association between non-WBC-reduced ABT and postoperative infection [summary odds ratio (OR) = 1·38, 95% confidence interval (CI) 1·03–1·85, $P < 0·05$; and summary OR = 1·56, 95% CI 1·06–2·31, $P < 0·05$, respectively]. When 12 RCTs reported up to 2005 were integrated despite extreme heterogeneity, both intention-to-treat and as-treated comparisons found no association of non-WBC-reduced ABT with postoperative infection (summary OR = 1·24, 95% CI 0·98–1·56, $P > 0·05$; and summary OR = 1·31, 95% CI 0·98–1·75, $P > 0·05$, respectively). In both analyses, the separate integration of four RCTs transfusing red blood cells (RBCs) or whole blood filtered after storage showed an association between non-WBC-reduced ABT and postoperative infection, whereas the separate integration of six (or nine) RCTs, reported through 2002 or 2005, and transfusing prestorage-filtered RBCs showed no association, whether intention-to-treat or as-treated comparisons were used. Thus, the published meta-analyses have produced discordant results because they did (or did not) investigate medical sources of heterogeneity and did (or did not) include the most recent RCTs. Intention-to-treat and as-treated comparisons produced concordant results.

Key words: transfusion-related immunomodulation; postoperative infection; white-blood-cell reduction; leucoreduction; meta-analysis.

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The debate over the existence of a causal relationship between non-white-blood-cell (WBC)-reduced allogeneic blood transfusion (ABT) and postoperative bacterial infection has been long and sometimes acrimonious [1–4]. Initially, the debate focused on differing interpretations of the findings of approximately 40 observational studies that had compared the risk of postoperative bacterial infection between transfused and untransfused patients undergoing gastrointestinal surgery, orthopaedic operations, cardiac surgery, or various other procedures. These studies tended to indicate that patients receiving perioperative transfusion (compared to those not receiving transfusion) almost always had a higher risk of developing postoperative bacterial infection [1]. The studies also indicated that patients receiving ABT differed from those not receiving transfusion in several prognostic factors that predisposed to adverse clinical outcomes [2]. Based on these two sets of observations, some authors concluded that ABT has a clinically manifest deleterious effect on the recipient, causing an increased risk of postoperative bacterial infection [1,3]. Other investigators concluded that need for ABT can be a surrogate marker for a variety of adverse prognostic factors and that these other variables that generated the need for ABT in the published studies probably also determined the subsequent clinical outcome [2,4].

Today, the controversy is focused on the differing interpretations [3,4] of the findings of the available randomized controlled trials (RCTs) that compared the risk of postoperative infection between recipients of non-WBC-reduced vs. WBC-reduced allogeneic red blood cells (RBCs). Three meta-analyses [5–7] of these RCTs have produced discordant results. Fergusson *et al.* [5] and Blumberg *et al.* [6] integrated the results of all RCTs published or reported up to 2002, and they calculated a reduction in the risk of postoperative infection attributable to WBC reduction. Vamvakas [7] did not find an association between non-WBC-reduced (compared with WBC-reduced) ABT and an increased risk of postoperative infection when he integrated the results of medically and statistically homogeneous subsets of RCTs published or reported up to 2005, except for a deleterious ABT effect manifest across studies that had transfused to the WBC-reduced arm allogeneic RBCs filtered after storage.

Fergusson *et al.* [5,8], Blumberg *et al.* [6], and Kunz and Guyatt [9] attributed the discordant findings of the meta-analyses to the integration of results of RCTs based on as-treated analyses in the meta-analyses of Fergusson *et al.* [5] and Blumberg *et al.* [6] vs. the integration of results of RCTs based on intention-to-treat analyses in the meta-analysis of Vamvakas [7]. In RCTs of ABT and postoperative infection, patients meeting the eligibility criteria for each trial were randomly assigned by the investigators, before their operation, to receive non-WBC-reduced vs. WBC-reduced ABT in the event that they needed perioperative transfusion. However, the need for perioperative transfusion cannot always be predicted preoperatively. Thus, some of the randomized patients in either arm of each trial ended up receiving perioperative ABT, in accordance with the transfusion criteria employed at the institution where they underwent surgery, because, based on the imperfect and incomplete data that are available during surgery in order to make this sort of judgement, their surgeons determined that the local transfusion criteria had been met and therefore the patients needed ABT. Other randomized subjects were not deemed by their surgeons to need (and thus they did not receive) transfusion.

Intention-to-treat analyses retained in the analysis all patients who had been randomized to receive non-WBC-reduced vs. WBC-reduced ABT, regardless of whether the patients ended up receiving perioperative ABT or not. In contrast, as-treated analyses withdrew from the analysis patients who had been randomized to receive non-WBC-reduced vs. WBC-reduced ABT if these subjects did not end up receiving perioperative ABT; and they relied on a comparison of two subgroups: the transfused patients who had been randomized to receive non-WBC-reduced ABT in the event that they might need ABT, vs. the transfused patients who had been randomized to receive WBC-reduced ABT in the event that they might need ABT. When many randomized subjects end up not receiving perioperative ABT, the power of the analysis to detect a deleterious effect of non-WBC-reduced ABT can often be significantly increased if as-treated (as opposed to intention-to-treat) analyses are used. For this reason, Fergusson *et al.* [5,8], Blumberg *et al.* [6] and Kunz and Guyatt [9] advocated that RCTs and meta-analyses of RCTs investigating the association between non-WBC-reduced (vs. WBC-reduced) ABT and postoperative infection should rely on as-treated (as opposed to intention-to-treat) comparisons.

The drawback of reduced power of intention-to-treat analyses in detecting a deleterious effect of non-WBC-reduced ABT is acknowledged by all. However, this disadvantage of an intention-to-treat analysis can be remedied by increasing the sample size of a study, and it is thus more of a concern at the level of an individual RCT than at the level of a meta-analysis of RCTs. Presently, more than 6000 patients (including more than 4000 transfused patients) have been enrolled in RCTs of the association between non-WBC-reduced ABT and postoperative infection. When such a large sample of patients is available and some 50% of the subjects receive ABT, a meta-analysis has sufficient power to detect a clinically relevant ABT effect whether intention-to-treat or as-treated analyses are employed. Accordingly, meta-analysts should be more concerned about maintaining the validity of their findings than about avoiding any dilution of the ABT effect that may result from the inclusion of patients not receiving transfusion in an intention-to-treat analysis.

Kunz and Guyatt [9] concluded by stating: 'Because it leads to an unbiased estimate, researchers and methodologists have reached a consensus that observing the intention-to-treat

principle is the superior approach. In *some* transfusion studies, however, it is justifiable to remove patients from the analysis after they have been randomized. The superiority of intention-to-treat (compared with as-treated) analyses of RCTs [10–16] had been established long before the controversy about an association between non-WBC-reduced ABT and postoperative infection started, although the consensus on the imperative for adherence to the intention-to-treat principle had never been absolute [17,18]. The recent pronouncements that 'the imperatives of the scientific method and the intention-to-treat principle can be conflicting' [6], however, may lead some investigators conducting RCTs of transfusion therapies not to adhere to the intention-to-treat principle, with a potential to generate biased results.

The three available meta-analyses [5–7] of the RCTs of the association between non-WBC-reduced (vs. WBC-reduced) ABT and postoperative infection differed in at least three key characteristics: (i) the investigation of the pertinent sources of medical heterogeneity among the included RCTs [19,20] and the integration (or not) of the results of RCTs despite extreme heterogeneity; (ii) the reliance on intention-to-treat vs. as-treated comparisons; and (iii) the number and year of publication of the RCTs included in the meta-analysis. Therefore, it cannot be taken for granted that the discrepant results between the three analyses were due to adherence (vs. non-adherence) to the intention-to-treat principle. Moreover, whether RCTs of transfusion therapies must (or need not) adhere to the intention-to-treat principle is a topic with much more far-reaching implications for transfusion medicine research than the debate on whether non-WBC-reduced ABT is causally related to postoperative bacterial infection.

For these reasons, the present study was undertaken to consider whether: (i) the disagreements between the three available meta-analyses are indeed due to reliance on intention-to-treat [7] vs. as-treated [5,6] analyses; and (ii) under what conditions (if any) it may be reasonable for RCTs of the association between non-WBC-reduced ABT and postoperative infection to deviate from to the intention-to-treat principle.

Investigation of medical sources of heterogeneity and integration of results of studies despite extreme heterogeneity

Table 1 lists the component parts of a meta-analysis of RCTs investigating the association between non-WBC-reduced ABT and postoperative infection. Meta-analysts should calculate a summary odds ratio (OR) across all available studies only when the reported RCTs are deemed (or found) to be medically and statistically homogeneous. In the absence of such homogeneity, meta-analysts should calculate summary ORs only across homogeneous subsets of studies that can be reasonably considered to target an ABT effect that is biologically the same. Such analyses of homogeneous subsets often help explain the discrepant results of available RCTs.

Table 2 shows the design attributes of the 12 RCTs [21–32] that examined the association of non-WBC-reduced ABT with an increased risk of postoperative infection between 1 January 1992 and 31 December 2005 [7]. The risk of postoperative infection has varied from a 7·3-fold increase in association with non-WBC-reduced (compared to WBC-reduced) ABT to no ABT effect (Fig. 1, part A). With one exception [29], however, all studies reported after 1998 [26–32] have reported negative findings.

These 12 RCTs differed in the RBC product transfused to the non-WBC-reduced arm, the RBC product transfused to the WBC-reduced arm, and/or the surgical setting (Table 2).

Table 1 Component parts of a meta-analysis of RCTs of the association between non-WBC-reduced ABT and postoperative infection

1. Retrieval of all reported RCTs of the hypothesis of increased risk of postoperative bacterial infection in association with non-WBC-reduced (compared to WBC-reduced) ABT
2. Extraction of data from each study on the effect of the exposure to non-WBC-reduced (compared with WBC-reduced) ABT
3. Assessment of whether the available reports are sufficiently similar to each other in their design (i.e. medically homogeneous) to be combined in a meta-analysis: assessment of their similarity in factors related to the exposure under study (receipt of non-buffy-coat-reduced vs. buffy-coat-reduced non-WBC-reduced allogeneic RBCs or whole blood; receipt of WBC-reduced allogeneic RBCs or whole blood filtered before vs. after storage), the clinical setting in which the hypothesis of interest is evaluated, and the outcome under study (e.g. diagnostic criteria for and frequency of postoperative infection)
4. Assessment of whether the results of the available reports are sufficiently in agreement with one another (i.e. statistically homogeneous) to be combined in a meta-analysis

Followed by either:

5a. When the available studies are medically and/or statistically heterogeneous, examination of the reasons for the disagreements between the studies: calculation of a 'summary' (or 'average') estimate(s) of the effect(s) of the exposure to non-WBC-reduced ABT across medically and statistically homogeneous subsets of studies

or

5b. When all studies are found to be medically and statistically homogeneous, integration of the results of all individual RCTs: calculation of a 'summary' (or 'average') estimate of the effect of the exposure to non-WBC-reduced ABT across all available studies

Table 2 Attributes of RCTs investigating the association of non-WBC-reduced ABT with postoperative infection

Study	Clinical setting	Number of randomized patients	Number of transfused patients (%)	Double blind	Multi-centre	RBC product given to the non-WBC-reduced arm	RBC product given to the WBC-reduced arm	Percentage of patients developing postoperative infection	
								Randomized patients	Transfused patients
Jensen *et al.* [21]	Colorectal surgery	197	104 (52·8)	No	No	Allogeneic whole blood	Allogeneic whole blood filtered after storage	8·1	15·4
Houbiers *et al.* [22]	Colorectal cancer resection	697	446 (64·0)	No	Yes	Buffy-coat-reduced allogeneic RBCs	Allogeneic RBCs filtered before storage	33·4	38·8
Jensen *et al.* [23]	Colorectal surgery	586	260 (44·4)	No	No	Buffy-coat-reduced allogeneic RBCs	Allogeneic RBCs filtered after storage	21·0	32·7
van de Watering *et al.* [24]	Cardiac surgery	914	866 (94·7)	No	No	Buffy-coat-reduced allogeneic RBCs	Allogeneic RBCs filtered before ($n = 305$) or after ($n = 303$) storage	19·3	19·7
Tartter *et al.* [25]	Gastrointestinal surgery	221	59 (26·7)	No	No	Allogeneic RBCs	Allogeneic RBCs filtered after storage	16·7	32·2
Titlestad *et al.* [26]	Colorectal surgery	279	112 (40·1)	Yes	No	Buffy-coat-reduced allogeneic RBCs	Allogeneic RBCs filtered before storage	30·1	42·0
Bilgin *et al.* [29]	Cardiac surgery	474	430 (90·7)	Yes	Yes	Buffy-coat-reduced allogeneic RBCs	Allogeneic RBCs filtered before storage	28·9	29·1
Wallis *et al.* [27]	Cardiac surgery	597	509 (85·3)	No	No	Buffy-coat-reduced ($n = 204$) or plasma-reduced[a] ($n = 198$) allogeneic RBCs	Allogeneic RBCs filtered before storage	13·7	14·5
van Hilten *et al.* [28]	Acute ($n = 79$) or elective ($n = 413$) aortic aneurysm repair; resection of gastrointestinal malignancy	1052	479 (45·5)	Yes	Yes	Buffy-coat-reduced allogeneic RBCs	Allogeneic RBCs filtered before storage	23·2	35·7
Bracey *et al.* [30]	Cardiac surgery	443	365 (82·4)	No	No	Allogeneic RBCs	Allogeneic RBCs filtered before storage	14·4	17·5
Boshkov *et al.* [31]	Cardiac surgery	562[b]	562 (100·0)	Yes	No[c]	Allogeneic RBCs	Allogeneic RBCs filtered before storage	19·6	19·6
Nathens *et al.* [32]	Trauma patients	268[b]	268 (100·0)	Yes	No	Allogeneic RBCs stored for < 25 days	Allogeneic RBCs filtered before storage	33·2	33·2

[a]In terms of its WBC content, this component is equivalent to non-buffy-coat-reduced allogeneic RBCs.

[b]Transfused patients only (as opposed to all randomized subjects): see text.

[c]Although patients admitted to three hospitals were enrolled, the same surgical team operated upon all patients.

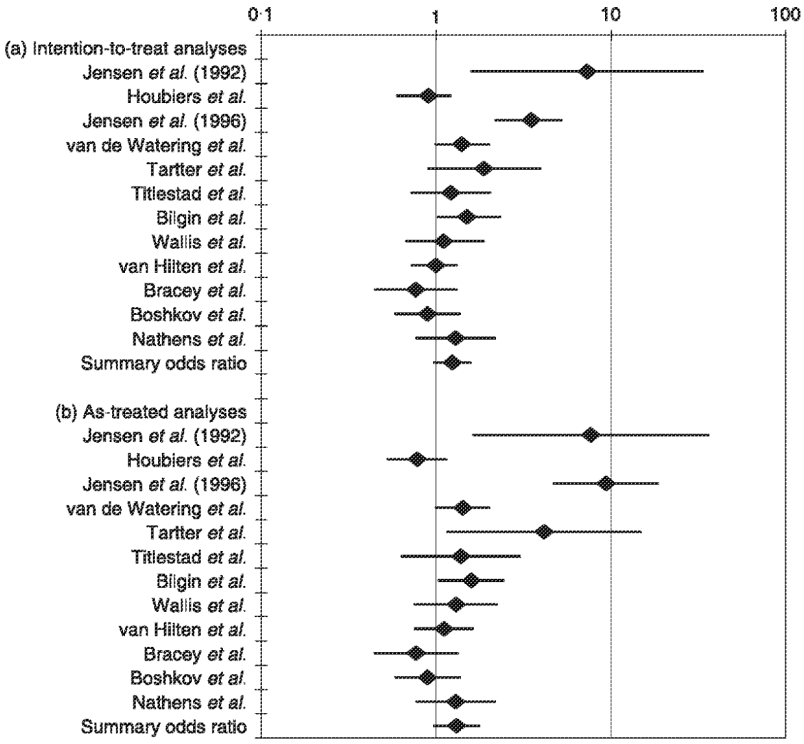

Fig. 1 RCTs investigating the association of non-WBC-reduced ABT with postoperative infection [21–32]. (a) For each RCT, the odds ratio (OR) of postoperative infection in recipients of non-WBC-reduced vs. WBC-reduced allogeneic RBCs or whole blood, as calculated from an intention-to-treat analysis. (b) For each RCT, the OR of postoperative infection in recipients of non-WBC-reduced vs. WBC-reduced allogeneic RBCs or whole blood, as calculated from an as-treated analysis. Each OR is surrounded by its 95% confidence interval (CI). If the 95% CI of the OR includes the null value of 1, there is no association between non-WBC-reduced ABT and postoperative infection ($P > 0{\cdot}05$). An association between non-WBC-reduced ABT and postoperative infection ($P < 0{\cdot}05$) is indicated by an OR > 1, provided that the associated 95% CI does not include the null value of 1. For each of the two analyses, the figure also shows a summary OR calculated across all 12 RCTs in the presence of extreme medical and statistical heterogeneity (see text). For the references to the listed studies, see Table 2.

Nine RCTs [22,24,26–32], including all RCTs published or reported after 1998, transfused to the WBC-reduced arm allogeneic RBCs filtered before storage (Table 2). Thus, for patients in the WBC-reduced arm, these RCTs [22,24,26–32] abrogated both the ABT effects mediated by immunologically competent allogeneic mononuclear cells [33–35] and the ABT effects mediated by WBC-derived soluble mediators that progressively accumulate in the supernatant fluid of RBCs during storage [36–39]. In contrast, four RCTs published between 1992 and 1998 [21,23–25] transfused to the WBC-reduced arm allogeneic RBCs or whole blood filtered after storage. For patients in the WBC-reduced arm, these RCTs [21,23–25] prevented effects mediated by immunologically competent allogeneic mononuclear cells [33–35], but not effects mediated by WBC-derived soluble mediators that accumulate during storage [36–39].

Five RCTs [24,27,29–31] were conducted in cardiac surgery and five [21–23,25,26] in gastrointestinal surgery. The ABT effect may be enhanced in the setting of cardiac surgery, because WBC-derived soluble mediators and/or allogeneic mononuclear cells may act as a second inflammatory insult, compounding the diffuse inflammatory response to the extracorporeal circuit and predisposing to postoperative complications [40]. Alternatively, the ABT effect may be enhanced in the 'unclean' setting of gastrointestinal surgery. Either way, it is possible for a deleterious ABT effect to become manifest only in the presence of co-factors, such as the special conditions that exist in cardiac or gastrointestinal surgery.

In addition, there was great variation among the RCTs in the amount of blood transfused and the frequency of a diagnosis of postoperative infection (Table 2). As few as 26·7% of randomized subjects needed perioperative transfusion in some gastrointestinal surgery studies [25]; in contrast, as many as 94·7% of randomized subjects needed perioperative transfusion in some cardiac surgery studies [24]. In gastrointestinal surgery, the frequency of postoperative infection ranged from 8·1 to 33·4% [21,22]. The differences in the proportion of transfused patients reflected patient-related selection factors (severity of underlying illness) as well as setting- and surgeon-related selection factors (subjective application of liberal or conservative transfusion criteria during an operation when objective laboratory indicators of the need for transfusion are not available). The differences in the frequency of postoperative infection reflected differences in the patients' severity of illness and the diagnostic criteria for infection, differences in the types of infections evaluated in each study, and perhaps also the effects of observation and/or selection bias [as not all RCTs were double blind and, in most cases, the details of the randomization procedure(s) were not reported].

Thus, it is most unlikely that all 12 RCTs targeted an increase in the risk of postoperative infection mediated by a deleterious ABT effect that was biologically the same in all cases. Instead, these RCTs most likely targeted effects of non-WBC-reduced ABT that differed both in magnitude and in nature – being mediated by either allogeneic mononuclear

cells [33–35] or WBC-derived soluble mediators [36–39], or both, and being compounded (or not) by other co-factors (such as a diffuse inflammatory response to the extracorporeal circuit). Accordingly, a meta-analysis integrating the results of all 12 available studies would not establish an effect attributed to a specific biologic mediator or specific biologic mechanism. Stated in other words, the medical heterogeneity of the available RCTs made it inappropriate to combine the results of all 12 RCTs in a meta-analysis [41–43].

The extreme medical heterogeneity of the studies was also reflected in extreme statistical heterogeneity: the probability that the variation in the results of the 12 RCTs might have arisen by chance was less than 1% ($P < 0{\cdot}01$ for the Q-test statistic), offering a further reason why it would be inappropriate to combine the findings of all 12 RCTs in a meta-analysis [41–43].

In fact, meta-analyses of clinically homogeneous subsets of RCTs that reported on postoperative infection (Table 2) produced results diametrically opposed to the findings expected from the theory that attributes the effect of non-WBC-reduced ABT to WBC-derived soluble mediators [36–39]: there was a reduction in the risk of postoperative infection in association with poststorage (but not prestorage) WBC reduction [7]. More specifically, across nine relatively homogeneous RCTs [22,24,26–32] that transfused allogeneic RBCs filtered before storage to the WBC-reduced arm, no increase in the risk of postoperative infection was detected in association with non-WBC-reduced ABT [summary OR = 1·06, 95% confidence interval (CI) 0·91–1·24; $P > 0{\cdot}05$] [7]. If the ABT effects were mediated by WBC-derived soluble mediators, prestorage filtration should have abrogated an increased infection risk associated with non-WBC-reduced ABT, because it would have removed the allogeneic WBCs from the components given to the WBC-reduced arm before the WBCs could release any significant amounts of mediators into the supernatant fluid.

In contrast, across four RCTs [21,23–25] that transfused RBCs filtered after storage to the WBC-reduced arm, there was a more than two-fold increase in the risk of infection in association with non-WBC-reduced ABT (summary OR = 2·25, 95% CI 1·12–4·25, $P < 0{\cdot}05$). If the ABT effects were mediated by WBC-derived soluble mediators, poststorage filtration should not have abrogated an increased infection risk associated with non-WBC-reduced ABT, because it would not have removed such mediators from the supernatant fluid of the stored RBCs given to the WBC-reduced arm of the studies.

Intention-to-treat vs. as-treated analyses

Figure 1a shows the 12 RCTs [21–32] that had compared recipients of non-WBC-reduced vs. WBC-reduced allogeneic RBCs or whole blood and had reported on the risk of postoperative infection by 31 December 2005. The results are shown either as presented by the authors or recalculated based on an intention-to-treat analysis. As already discussed, there was extreme medical heterogeneity among these RCTs that was also reflected in extreme statistical heterogeneity ($P < 0{\cdot}01$ for the Q-test statistic) [7]. Nonetheless, if the results of all 12 RCTs were to be combined under such conditions of extreme heterogeneity by the random-effects method of DerSimonian and Laird [44], the effect of non-WBC-reduced ABT on postoperative infection would not attain statistical significance (summary OR = 1·24, 95% CI 0·98–1·56, $P > 0{\cdot}05$) (Fig. 1a).

Figure 1b shows these same RCTs [21–32], but the results are now shown either as presented by the authors or recalculated based on a as-treated analysis. The medical heterogeneity among the studies is the same as before, but it is now reflected in even greater statistical heterogeneity ($P < 0{\cdot}001$ for the Q-test statistic). The reason for the greater statistical heterogeneity is that the results of three of the 12 RCTs [21,23,25] show a much larger effect of non-WBC-reduced ABT if they are analysed using as-treated comparisons. The results of these three RCTs [21,23,25] differ greatly from the results of the remaining RCTs (Fig. 1a), and the difference between their results and those of the remaining RCTs is further accentuated when as-treated comparisons are used (Fig. 1b). Nonetheless, if the results of all 12 RCTs (analysed based on as-treated comparisons) were to be combined under such conditions of extreme heterogeneity, the effect of non-WBC-reduced ABT on postoperative infection would not attain statistical significance (summary OR = 1·31, 95% CI 0·98–1·75, $P > 0{\cdot}05$).

Table 3 shows the results of meta-analyses investigating possible sources of variation in the findings of RCTs that examined the association between non-WBC-reduced ABT and postoperative infection. Both intention-to-treat and as-treated meta-analyses are shown, and there is agreement between the findings of the intention-to-treat and as-treated meta-analyses in all cases. The effect of WBC-containing ABT on postoperative infection attains significance ($P < 0{\cdot}05$) only when the results of RCTs transfusing to the WBC-reduced arm allogeneic RBCs or whole blood filtered after storage are integrated separately. When these four RCTs [21,23–25] are combined, the risk of postoperative infection in association with WBC-containing ABT is more than doubled in the intention-to-treat analysis and more than quadrupled in the as-treated analysis (summary OR = 2·25, 95% CI 1·12–4·25, $P < 0{\cdot}05$; and summary OR = 4·05, 95% CI 1·09–15·10, $P < 0{\cdot}05$, respectively).

In all other subgroup analyses, neither the intention-to-treat nor the as-treated meta-analysis detects a statistically significant association between non-WBC-reduced ABT and postoperative infection. In particular, in the case of the nine homogeneous RCTs [22,24,26–32] that transfused to the WBC-reduced arm allogeneic RBCs filtered before storage, the findings of the two meta-analyses are virtually identical (summary OR = 1·06, 95% CI 0·91–1·24, $P > 0{\cdot}05$; and summary OR = 1·09, 95% CI 0·91–1·32, $P > 0{\cdot}05$, respectively).

Table 3 Medical sources of variation in the findings of RCTs investigating the association between non-WBC-reduced ABT and postoperative infection

Study attribute	Categories of study attribute	Number of studies [references]	Number of patients included in the analysis		*Q*-test statistic: *P* value		Summary odds ratio (95% CI)	
			Intention-to-treat	As-treated	Intention-to-treat	As-treated	Intention-to-treat	As-treated
RBC product transfused to the non-WBC-reduced arm	Non-buffy-coat-reduced allogeneic RBCs or whole blood	6 [21,25,27,30–32]	2084	1692	= 0·10	< 0·025[a]	1·17 (0·83–1·67)	1·26 (0·81–1·96)
	Buffy-coat-reduced allogeneic RBCs	7 [22–24,26–29]	4403	2944	< 0·01[a]	< 0·001[a]	1·26 (0·93–1·72)	1·35 (0·91–2·01)
RBC product transfused to the WBC-reduced arm	Allogeneic RBCs filtered before storage	9 [22,24,26–32]	4982	3757	> 0·20	= 0·20	1·06 (0·91–1·24)	1·09 (0·91–1·32)
	Allogeneic RBCs or whole blood filtered after storage	4 [21,23–25]	1616	1006	= 0·05	< 0·01[a]	2·25[b] (1·12–4·25)	4·05[b] (1·09–15·10)
Surgical setting	Cardiac surgery	5 [24,27,29–31]	2990	2732	> 0·20	> 0·20	1·13 (0·87–1·46)	1·15 (0·88–1·52)
	Gastrointestinal surgery	5 [21–23,25,26]	1983	981	< 0·001[a]	< 0·001[a]	1·75 (0·89–3·42)	2·76 (0·80–9·52)

[a]Results integrated under conditions of heterogeneity ($P < 0·05$ for the *Q*-test statistic).
[b]Statistically significant summary OR ($P < 0·05$) calculated from both the as-treated and the intention-to-treat analyses.

Thus, when all 12 RCTs available today are considered together in a meta-analysis, the type of analysis used (intention-to-treat vs. as-treated) does not explain the discordant findings reported by the three meta-analyses [5–7]. In contrast, there is agreement between the results of the intention-to-treat and as-treated analyses. Therefore, the controversy whether intention-to-treat or as-treated analyses should be used [3–9] is no longer relevant to the debate whether non-WBC-reduced ABT is (or is not) causally related to postoperative bacterial infection.

Despite this, the controversy whether intention-to-treat or as-treated analyses should be used [3–9] is hardly of just academic relevance to transfusion medicine. In fact, it is crucial for the future of clinical research that the reasons why it is important to adhere to the intention-to-treat principle be understood by researchers and clinicians alike; and that it also be understood that deviations from this principle may be reasonable only in specific situations where other conditions are met to ensure that non-adherence to the intention-to-treat principle will not bias the results.

The intention-to-treat principle dictates that no withdrawals of patients be allowed after the randomization step, and that all randomized subjects be analysed within the arm to which they were randomly assigned, regardless of how much (or how little) treatment they actually received [10–17]. Therefore, patients randomized to receive non-WBC-reduced or WBC-reduced ABT (in the event that they needed perioperative transfusion) [21–32] had to be analysed within the non-WBC-reduced or WBC-reduced arm, regardless of whether they received transfusion or not.

The purpose of randomization is to prevent confounding variables (associated with both the need for transfusion and the risk of postoperative infection and occurring differentially in one vs. the other arm of the RCT) from generating a spurious association between non-WBC-reduced ABT and postoperative infection. By assigning subjects to arms randomly, randomization ensures that, in terms of the levels of both known and unknown confounders, the entire arm of subjects randomized to receive non-WBC-reduced ABT is equivalent to the entire arm of subjects randomized to receive WBC-reduced ABT. This does not mean, however, that the transfused patients from the non-WBC-reduced arm are also equivalent to the transfused patients from the WBC-reduced arm. In fact, because the subjects who did not need perioperative transfusion represent the least sick patients rather than a random subset of the randomized subjects, the transfused patients from the non-WBC-reduced arm may well differ from the transfused patients from the WBC-reduced arm in the levels of known and/or unknown confounding factors.

Kunz and Guyatt [9] stated that 'in *some* transfusion studies, it is justifiable to remove patients from the analysis after they have been randomized'. The rationale for this opinion was that 'as long as the decision to transfuse is uninfluenced by

the patient's allocation to WBC-reduced or non-WBC-reduced ABT ... one is in effect removing patients *at random; that being the case*, groups that were prognostically balanced by random allocation will remain balanced.'

Although this rationale has merit in some clinical situations, it cannot be applied unconditionally to all RCTs of the association between non-WBC-reduced ABT and postoperative infection (Table 2). Random removal of patients means that each randomized subject has an equal chance of being removed from an arm of an RCT. However, in these transfusion studies, the removal of untransfused patients did not occur randomly, but because of selection factors, as some patients had less severe illness and were judged by their surgeons not to need transfusion.

If (i) the RCTs subjected to as-treated analyses had been double blind (precluding the possibility that the decision to transfuse was influenced by a patient's allocation to the non-WBC-reduced or WBC-reduced arm); and (ii) the decision to transfuse RBCs (in each of the perioperative settings in which these RCTs were conducted) had been based on objective and consistent data; one could consider that the patient/surgeon selection factors that resulted in some patients' not being transfused in the non-WBC-reduced arm of each RCT could have been the mirror image of the selection factors that resulted in some patients' not being transfused in the WBC-reduced arm of that RCT. Had this been the case, the two groups could have remained balanced after the untransfused subjects were withdrawn.

However, several of the RCTs subjected to as-treated analyses were not double blind (Table 2); also, the decision to transfuse RBCs during an operation is, at least in part, based on the surgeon's impression of whether a patient needs to be transfused, rather than on reliable clinical indicators that are available when needed. Even when surgeons adhere to objective criteria when possible, the values that determine whether the transfusion criteria have been met are often unavailable or unreliable during surgery. Thus, to accept the rationale of Gunz and Guyatt [9], one must accept that, because the decision to transfuse did not, presumably, depend on the arm to which a patient had been allocated, the same clinical impressions must have been engendered (thereby resulting in the transfusion of some patients and the withdrawal of transfusion from other patients) when varying surgical teams operated on patients with a 'borderline' need for transfusion who had been allocated to either the non-WBC-reduced or the WBC-reduced arm of each study.

In this author's opinion, such an assumption could perhaps be made if all patients included in an RCT were operated upon by the same surgeon, or at least by the same surgical team (consisting of individuals who adhere, consciously and subconsciously, to the same, liberal or conservative, transfusion criteria). Such an assumption cannot be made in multicentre studies, while the willingness to make it in single-centre studies is largely a matter of faith. Only in the study of Tartter *et al.* [25], in which all patients were operated upon by the same surgeon, could one argue that this assumption *should* be made.

Because the RCTs of Boshkov *et al.* [31] and Nathens *et al.* [32] were both double blind and single centre, Vamvakas [7] considered that, in these studies, the transfused patients from the non-WBC-reduced arm could be the mirror image of the transfused patients from the WBC-reduced arm in terms of the distribution of confounding factors that predispose to both the need for transfusion and the risk of infection. Accordingly, Vamvakas [7] integrated the results from the as-treated analyses presented by these two studies [31,32] with the findings from the intention-to-treat analyses from all other RCTs [21–30]. Table 2 shows that this assumption could also be made for the study of Titlestad *et al.* [26], which was also double blind and single centre, but it could not be made a priori for the remaining nine RCTs.

Importantly, this assumption could not be made for the studies of Jensen *et al.* [21,23] and Tartter *et al.* [25] that were not double blind (Table 2). As already discussed, these are the only studies for which the results from as-treated analyses deviate from the findings of intention-to-treat comparisons (compare the results of these studies in Fig. 1a and b). These are also the only studies whose inclusion in meta-analyses causes the results of as-treated meta-analyses to differ, at least as far as the magnitude of the calculated summary OR is concerned (compare the ORs from the meta-analyses of the gastrointestinal-surgery RCTs and of the RCTs transfusing poststorage-filtered allogeneic RBCs to the WBC-reduced arm in Table 3). Arithmetic differences in the calculated ORs aside, however, it should be borne in mind that both the intention-to-treat and the as-treated meta-analyses produced concordant results as far as statistical significance is concerned (Table 3).

Number and year of publication of studies included in the meta-analysis

The third possible reason why the available meta-analyses produced discordant results is that they relied on analyses of different combinations of RCTs of the association between non-WBC-reduced ABT and postoperative infection. At least in part, this difference in the number and year of publication of the included studies reflected which RCTs were available at the time that each meta-analysis was undertaken [5,7]. In other cases, it reflected the decision of the meta-analysts not to include data from RCTs not yet published in full, because RCTs not yet published in full cannot be subjected to a detailed qualitative assessment [6]. This is a valid position; however, because the most recent RCTs are generally the ones not yet published in full, it resulted in the exclusion of the three most recent RCTs [29–32] from the report of Blumberg *et al.* [6].

To investigate whether the disagreements between the available RCTs were due to the different combinations of RCTs included in each meta-analysis, the analyses shown in Fig. 1 were restricted to a subgroup analysis of the nine initial RCTs [21–29] that had been included in the report of Blumberg *et al.* [6]. The data presented by Blumberg *et al.* [6] were checked to ensure that the same input data as those used in that report [6] were used here as well for the as-treated analysis of each RCT. As far as these nine studies [21–29] are concerned, the only difference between the input data used here and the input data used by Blumberg *et al.* [6] pertained to the RCT of Wallis *et al.* [27] that had used two arms receiving non-WBC-reduced RBCs (Table 2). Blumberg *et al.* [6] included in their analysis of patients receiving non-WBC-reduced ABT only the data on recipients of plasma-reduced allogeneic RBCs ($n = 158$). Here, the data pertaining to recipients of both plasma-reduced and buffy-coat-reduced ($n = 333$) allogeneic RBCs make up the non-WBC-reduced arm of recipients of non-WBC-reduced ABT in the as-treated analysis of that trial [27].

An as-treated meta-analysis of these nine RCTs [21–29] calculated a statistically significant association between non-WBC-reduced ABT and increased risk of postoperative infection (summary OR = 1·56, 95% CI 1·06–2·31, $P < 0·05$); that is, an increase in the risk of infection in the non-WBC-reduced group by 56% – a finding similar to the previously reported reduction in the risk of infection in the WBC-reduced group by 47% (as calculated by Blumberg *et al.* [6]) or by 40% (as calculated by Fergusson *et al.* [5]). When the data from these same nine RCTs [21–29] were subjected to an intention-to-treat meta-analysis, a statistically significant association between non-WBC-reduced ABT and an increased risk of postoperative infection was again calculated (summary OR = 1·38, 95% CI 1·03–1·85).

However, in both the as-treated and the intention-to-treat meta-analyses, such a summary OR was calculated in the presence of extreme medical (Table 2) and statistical ($P < 0·001$ for the Q-test statistic) heterogeneity. For this reason, readers should resist the temptation to attribute a medical or biological meaning to the value of the calculated summary OR. These summary ORs are presented here solely for the purpose of illustration.

The main reason for the discordant findings of the three meta-analyses [5–7] has been the non-availability [5] or exclusion [6] of the data from the three latest RCTs [29–32] from the meta-analyses of Fergusson *et al.* [5] and Blumberg *et al.* [6] (although Fergusson *et al.* [6] did include the results from a subset of the patients of Bracey *et al.* [30] that were available at the time of their meta-analysis). Because it is the inclusion or exclusion of these three latest RCTs [29–32] that, for the most part, explains the disagreements between the three published meta-analyses [5–7], these three studies (Table 2) deserve further comment.

There were 1195 transfused patients in the three latest RCTs [29–32], and inclusion of these studies would have increased the total sample size of Blumberg *et al.* [6] from 3093 to 4288. Thus, the impact of these RCTs [29–32] on the results of the meta-analyses becomes readily apparent, as none of them had detected an association between non-WBC-reduced ABT and postoperative infection (Fig. 1). Importantly, two [31,32] of the three studies had reported only as-treated (as opposed to intention-to-treat) analyses, yet in neither case was there a significant association between non-WBC-reduced ABT and postoperative infection. All studies were single centre (Table 2), ruling out the possibility that a 'centre effect' might have diluted an actual ABT effect [45]. Moreover, two [31,32] of the three studies were double blind, and the transfused cellular blood components maximized the possibility of detection of a deleterious ABT effect: all three studies administered non-buffy-coat-reduced allogeneic RBCs to the non-WBC-reduced arm and allogeneic RBCs filtered before storage to the WBC-reduced arm.

The same sources of heterogeneity identified in the analysis of all 12 RCTs (Table 3) were also identified in the subgroup analysis of the nine initial RCTs (data not shown). All four RCTs that had transfused allogeneic RBCs filtered after storage to the WBC-reduced arm [21,23–25] were included in the analysis of Blumberg *et al.* [6]. The separate integration of the findings of these studies produced the results already reported in Table 3. Six RCTs that had transfused allogeneic RBCs filtered before storage to the WBC-reduced arm [22,24,26–29] were included in the analysis of Blumberg *et al.* [6]. The separate integration of the findings of these studies did not detect an association between non-WBC-reduced ABT and postoperative infection, whether results from intention-to-treat or as-treated comparisons were combined (summary OR = 1·12, 95% CI 0·92–1·36, $P > 0·05$; and summary OR = 1·18, 95% CI 0·93–1·50, $P > 0·05$, respectively).

Conclusions

This study investigated three possible reasons for the discordant findings of the meta-analyses of RCTs of non-WBC-reduced ABT and postoperative infection, and it found that there were two reasons for the disagreements between the findings of the published meta-analyses [5–7]: the investigation (or not) of pertinent sources of medical heterogeneity among the RCTs, along with the integration (or not) of findings of RCTs in the presence of extreme medical and statistical heterogeneity; and the inclusion in the meta-analysis of nine RCTs reported up to 2002 [21–29] or of 12 RCTs reported up to 2005 [21–32].

The use of intention-to-treat vs. untreated analyses had no effect on the results of the meta-analyses, whether the meta-analysis included all 12 RCTs presently available or

was limited to the nine initial RCTs included in the report of Blumberg *et al.* [6]. In the case of all 12 RCTs, neither the intention-to-treat nor the as-treated meta-analysis detected an association between non-WBC-reduced ABT and postoperative infection when results were integrated under conditions of extreme heterogeneity (Fig. 1). In the case of the nine RCTs, both the intention-to-treat and the as-treated meta-analyses detected a statistically significant ($P < 0{\cdot}05$) association between non-WBC-reduced ABT and postoperative infection when results were integrated under similar conditions of extreme heterogeneity.

Whether the authors investigated (or not) the pertinent sources of medical heterogeneity was an important reason for the disagreements between the meta-analyses [5–7]. Vamvakas [7] observed a big difference between the results produced by RCTs administering pre- vs. poststorage-filtered allogeneic RBCs to the WBC-reduced arm. Fergusson *et al.* [5] also noted the unusually large protective effect of bedside WBC reduction, but Blumberg *et al.* [6] did not investigate pre- vs. poststorage WBC reduction as a source of medical heterogeneity among the available RCTs, despite the fact that they did report on other possible sources of heterogeneity. In this author's opinion, the blood component administered to the WBC-reduced arm is the most important source of medical heterogeneity, because it directly reflects the postulated mechanism(s) of the ABT effect: an effect mediated by immunologically competent allogeneic mononuclear cells [33–35] and abrogated by either pre- or poststorage filtration; vs. an effect mediated by WBC-derived soluble mediators [36–39] and prevented only by prestorage filtration.

Thus, the deleterious ABT effect detected in the meta-analyses of the nine initial RCTs can be traced to three studies [21,23,25] that had administered components no longer used in modern transfusion practice. Although two [21,25] of these three studies had enrolled only small numbers of patients (especially when as-treated analyses were used – Table 2), their influence on the results of the meta-analyses is disproportionate to their size, because the random-effects method used here to integrate the results of the RCTs [44] gives more weight to small studies than would be warranted by their sample size. Importantly, two [21,23] of these three studies [21,23,25] had been conducted by the same team of Jensen *et al.* who administered allogeneic RBCs or whole blood filtered after storage to the WBC-reduced arm; and allogeneic whole blood to the non-WBC-reduced arm of their early study [21] (Table 2). Tartter *et al.* [25] similarly administered allogeneic RBCs filtered after storage to the WBC-reduced arm. Because bedside filtration is no longer used in North America and Europe, the results of these three studies conducted before 1998 [21,23,25] are probably no longer relevant to modern clinical transfusion practice.

With the current availability of 12 RCTs [21–32], it is no longer appropriate to rely on meta-analyses of medically heterogeneous studies to reach conclusions as to whether non-WBC-reduced ABT is (or is not) associated with postoperative bacterial infection. As a sufficient number of medically homogeneous RCTs have now been reported, public-policy decisions should be based on an integration of results of medically and statistically homogeneous studies. More specifically, it is now possible to rely on homogeneous subsets of RCTs that have transfused the same RBC product to both comparison arms. Six RCTs [22,24,26–29] transfused buffy-coat-reduced allogeneic RBCs to the non-WBC-reduced arm and prestorage-filtered allogeneic RBCs to the WBC-reduced arm (i.e. the blood components currently used in Europe). Four RCTs [27,30–32] transfused non-buffy-coat-reduced allogeneic RBCs to the non-WBC-reduced arm and prestorage-filtered allogeneic RBCs to the WBC-reduced arm (i.e. the blood components used in North America).

Current policy decisions regarding the use of WBC-reduced components in Europe or North America should be based on the results of the two groups of clinically homogeneous RCTs (Table 4). Policy decisions for all of the Western world should rely on the results of nine RCTs [22,24,26–32] that administered allogeneic RBCs filtered before storage to the WBC-reduced arm, the components used today in countries that have implemented universal WBC reduction and for selected patients in other countries. Meta-analyses of these nine RCTs (Table 3), or of the RCTs shown in Table 4, produce unequivocal results: whether based on as-treated or intention-to-treat comparisons, they detect no association between non-WBC-reduced ABT and postoperative infection.

Thus, the sometimes emotional debate over whether the RCTs of the association of non-WBC-reduced ABT and postoperative infection should undergo intention-to-treat vs. as-treated analyses should not divert attention from the importance of adhering to the intention-to-treat principle when RCTs or meta-analyses of RCTs of transfusion therapies are conducted. The purpose of RCTs is to ensure that conclusions are based on a comparison of two arms that are balanced in terms of the levels of all confounding factors; not two groups that may be so balanced. Meta-analysts cannot determine, from the report of an RCT, whether two groups restricted to the transfused subjects are balanced, because trialists do not report data on all known confounders; and because there are unknown (and/or poorly quantifiable) confounders for which only an analysis based on the intention-to-treat principle can ensure their balanced distribution between the comparison arms. Therefore, meta-analysts can integrate as-treated findings from RCTs only when other conditions are met to ensure that non-adherence to the intention-to-treat principle will not bias the results. In this author's opinion, as-treated results may be used by meta-analysts only if they are derived from double-blind RCTs in which all patients have been operated upon by the same surgical team.

Table 4 Meta-analyses of homogeneous subsets of RCTs transfusing the same RBC product to both comparison arms

RBC products transfused to the comparison arms of each trial	Number of studies [references]	Number of patients included in the analysis		*Q*-test statistic: *P* value		Summary OR (95% CI)	
		Intention-to-treat	As-treated	Intention-to-treat	As-treated	Intention-to-treat	As-treated
Non-buffy-coat-reduced allogeneic RBCs vs. WBC-reduced allogeneic RBCs filtered before storage	4 [27,30–32]	1666	1529	> 0·25	> 0·10	1·02 (0·77–1·35)	1·07 (0·75–1·52)
Buffy-coat-reducedd allogeneic RBCs vs. WBC-reduced allogeneic RBCs filtered before storage	6 [22,24,26–29]	3511	2404	> 0·10	> 0·10	1·09 (0·89–1·34)	1·12 (0·87–1·44)

References

1 Blumberg N, Heal JM: Effects of transfusion on immune function: cancer recurrence and infection. *Arch Pathol Lab Med* 1994; **118**:371–379

2 Vamvakas EC, Blajchman MA: Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? *Blood* 2001; **97**:1180–1195

3 Blumberg N: Deleterious clinical effects of transfusion immunomodulation: proved beyond a reasonable doubt. *Transfusion* 2005:**45** (Aug. Suppl.), 33S–39S

4 Vamvakas E: Deleterious clinical effects of transfusion immunomodulation: proved beyond a reasonable doubt. *Transfusion* 2006; **46**:492–494

5 Fergusson D, Khanna MP, Tinmouth A, Hébert PC: Transfusion of leukoreduced red blood cells may decrease postoperative infections: two meta-analyses of randomized controlled trials. *Can J Anaesth* 2004; **51**:417–424

6 Blumberg N, Zhao H, Wang H, Messing S, Heal JM, Lyman GH: The intention-to-treat principle in clinical trials and meta-analyses of leukoreduced blood transfusions in surgical patients. *Transfusion* 2007; **47**:573–581

7 Vamvakas EC: White-blood-cell-containing allogeneic blood transfusion and postoperative infection or mortality: an updated meta-analysis. *Vox Sang* 2007; **92**:224–232

8 Fergusson D, Aaron SD, Guyatt G, Hébert P: Post-randomisation exclusions: the intention-to-treat principle and excluding patients from analysis. *BMJ* 2002; **325**:652–654

9 Kunz R, Guyatt G: Which patients to include in the analysis? *Transfusion* 2006; **46**:881–884

10 Schwartz D, Lellouch J: Explanatory and pragmatic attitudes in therapeutic trials. *J Chronic Dis* 1967; **20**:637–648

11 Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. *Br J Cancer* 1976; **34**:585–612

12 Newell DJ: Intention-to-treat analysis: implications for quantitative and qualitative research. *Int J Epidemiol* 1992; **21**:837–841

13 Lee YJ, Ellenberg JH, Hirtz DG, Nelson KB: Analysis of clinical trials by treatment actually received: is it really an option? *Stat Med* 1991; **10**:1595–1605

14 Hollis S, Campbell F: What is meant by intention-to-treat analysis? Survey of published randomized controlled trials. *BMJ* 1999; **319**:670–674

15 Mahoer D, Schulz KF, Altman DG: The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *J Am Podiatr Med Assoc* 2001; **91**:437–442

16 Ruiz-Canella M, Martinez-Gonzalez MA, de Irala-Estevez J: Intention-to-treat analysis is related to methodological quality. *BMJ* 2000; **320**:670–674

17 Ellenberg JH: Intent-to-treat analysis versus as-treated analysis. *Drug Inf J* 1996; **30**:535–544

18 Sackett DL, Gent M: Controversy in counting and attributing events in clinical trials. *N Engl J Med* 1979; **301**:1410–1412

19 Petiti DB: Approaches to heterogeneity in meta-analysis. *Stat Med* 2001; **20**:3625–3633

20 Thompson SG: Why sources of heterogeneity in meta-analysis should be investigated. *BMJ* 1994; **309**:1351–1355
21 Jensen LS, Andersen AJ, Christiansen PM, Hokland P, Juhl CO, Madsen G, Mortensen J, Moller-Nielsen C, Hanberg-Sorensen F, Hokland M: Postoperative infection and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. *Br J Surg* 1992; **79**:513–516
22 Houbiers JGA, Brand A, van de Watering LMG, Hermans J, Verwey PJ, Bijnen AB, Pahlplatz P, Eeftinck Schattenkerk M, Wobbes T, de Vries JE: Randomized controlled trial comparing transfusion of leucocyte-depleted or buffy-coat-depleted blood in surgery for colorectal cancer. *Lancet* 1994; **344**:573–578
23 Jensen LS, Kissmeyer-Nielsen P, Wolff B, Quist N: Randomized comparison of leucocyte-depleted versus buffy-coat-poor blood transfusion and complications after colorectal surgery. *Lancet* 1996; **348**:841–845
24 van de Watering LMG, Hermans J, Houbiers JGA, van de Broeck PJ, Bouter H, Boer F, Harvey MS, Huysmans HA, Brand A: Beneficial effect of leukocyte depletion of transfused blood on post-operative complications in patients undergoing cardiac surgery: a randomized clinical trial. *Circulation* 1998; **97**:562–568
25 Tartter PI, Mohandas K, Azar P, Endres J, Kaplan J, Spivack M: Randomized trial comparing packed red cell blood transfusion with and without leukocyte depletion for gastrointestinal surgery. *Am J Surg* 1998; **176**:462–466
26 Titlestad IL, Ebbesen LS, Ainsworth AP, Lillevang ST, Quist N, Georgsen J: Leukocyte-depletion of blood components does not significantly reduce the risk of infectious complications: results of a double-blind, randomized study. *Int J Colorectal Dis* 2001; **16**:147–153
27 Wallis JP, Chapman CE, Orr KE, Clark SC, Forty JR: Effect of WBC reduction of transfused RBCs on postoperative infection rates in cardiac surgery. *Transfusion* 2002; **42**:1127–1134
28 van Hilten JA, van de Watering LMG, van Bockel JH, van de Velde CJH, Kievit J, Brand R, van den Hout WB, Geelkerken RH, Roumen RMH, Wesselink RMJ, Koopman-van Gemert A, Koning J, Brand A: Effects of transfusion with red cells filtered to remove leukocytes: randomized controlled trial in patients undergoing major surgery. *BMJ* 2004; **328**:1281–1284
29 Bilgin YM, van de Watering LMG, Eijsman L, Versteegh MIM, Brand R, van Oers MHJ, Brand A: Double-blind, randomized controlled trial on the effect of leukocyte-depleted erythrocyte transfusions in cardiac valve surgery. *Circulation* 2004; **109**:2755–2760
30 Bracey AW, Radovancevick R, Nussimeier NA, LaRocco M, Vaughn WK, Cooper JR: Leukocyte-reduced blood in open-heart surgery patients: effects on outcome. *Transfusion* 2002; **42** (Suppl.):5S
31 Boshkov LK, Furnary A, Morris C, Chien G, van Winkle D, Reik R: Prestorage leukoreduction of red cells in elective cardiac surgery: results of a double-blind randomized controlled trial. *Blood* 2004; **104**:112a
32 Nathens AB, Nester TA, Rubenfeld GD, Nirula R, Gernsheimer TB: The effects of leukoreduced blood transfusion on infection risk following injury: a randomized controlled trial. *Shock* 2006; **26**:342–347
33 Blajchman MA, Bardossy I, Carmen R, Sastry A, Singal DP: Allogeneic blood transfusion-induced enhancement of tumor growth: two animal models showing amelioration by leukodepletion and passive transfer using spleen cells. *Blood* 1993; **81**:1880–1882
34 Mincheff MS, Meryman HT, Kapoor V, Alsop P, Wotzel M: Blood transfusion and immunomodulation: a possible mechanism. *Vox Sang* 1993; **65**:18–24
35 Kao KJ: Induction of humoral immune tolerance to major histocompatibility complex antigens by transfusions of UV-B irradiated leukocytes. *Blood* 1996; **88**:4375–4382
36 Ghio M, Contini P, Mazzei C, Brenci S, Barbaris G, Filaci G, Indiveri F, Puppo F: Soluble HLA Class I, HLA Class II, and Fas ligand in blood components: a possible key to explain the immunomodulatory effects of allogeneic blood transfusion. *Blood* 1999; **93**:1770–1777
37 Bordin JO, Bardossy L, Blajchman MA: Growth enhancement of established tumors by allogeneic blood transfusion in experimental animals and its amelioration by leukodepletion: the importance of timing of the leukodepletion. *Blood* 1994; **84**:344–348
38 Nielsen HJ, Reimert CM, Pedersen AN, Brunner N, Edvarsen L, Dybkjaer E, Kehlet H, Skov PS: Time-dependent spontaneous release of white cell- and platelet-derived bioactive substances from stored human blood. *Transfusion* 1996; **36**:960–965
39 Innerhofer P, Luz G, Spotl L, Hobish-Hagen P, Schobersberger W, Fischer M, Nussbaumer W, Lochs A, Irschick E: Immunologic changes following transfusion of autologous or allogeneic buffy-coat-poor versus leukocyte-depleted blood in patients undergoing arthroplasty. I. Proliferative T-cell responses and T-helper/T-suppressor cell balance. *Transfusion* 1999; **39**:1089–1096
40 Fransen E, Maessen J, Denterner M, Senden N, Buurman W: Impact of blood transfusion on inflammatory mediator release in patients undergoing cardiac surgery. *Chest* 1999; **116**:1233–1239
41 *Diversity and Heterogeneity* (monograph on the Internet). Oxford: The Cochrane Collaboration; 2002, accessed 20 May 2006. Available from: http://www.cochrane-net.org/openlearning/HTML/mod13-5.htm
42 L'Abbé KA, Detsky AS, O'Rourke K: Meta-analysis in clinical research. *Ann Intern Med* 1987; **107**:224–233
43 Stein R: Meta-analysis from one FDA reviewer's perspective. *Proc Biopharmaceut Section Am Stat Assoc* 1988; **2**:34–38
44 DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**:177–188
45 Vamvakas EC: Multicenter randomized controlled trials in transfusion medicine. *Transfus Med Rev* 2000; **14**:137–150

