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TRANSFUSION

HOW DO I DO IT

How do I analyze the role of red blood cell transfusions on clinical outcome in anemic patients? Disentangling the effect of red blood cell transfusions from pre-operative anemia on post-operative outcome

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Abstract

Background: Preoperative anemia is associated with worse clinical postoperative outcomes and a higher risk of receiving red blood cell (RBC) transfusions. It is challenging to disentangle the effect of preoperative anemia from the effect of receiving RBC transfusions on postoperative clinical outcomes such as length of hospital stay (LOS). When analyzing the association of preoperative anemia on LOS, it is important to be able to analyze RBC transfusions as a mediator in this relationship. In this paper, the background and application of mediation analysis is outlined as a statistical methodology in transfusion medicine research.

Study Design and Methods: To explain the methodology of mediation analysis, a database from a previously reported clinical study was used (So-Osman C. et al. 2014) with anemia as the exposure variable and LOS as the primary outcome. Both the product-of-coefficients method and the change-in-coefficients method are used for mediation analysis, and linear regression models were used.

Results: In the example of a simplified analysis, two-thirds of the effect could be attributed to mediation. This result was obtained by both the product-of-coefficients method and the change-in-coefficients method.

Discussion: Mediation is assessed in a similar way as confounding, but the interpretation of the results is totally different. It is, therefore, of critical importance to distinguish between potential mediators and potential confounders in

Abbreviations: DAG, directed acyclic graph; Hb, hemoglobin; LOS, length of hospital stay; PBM, patient blood management; RBC, red blood cell.

transfusion research. Since the calculation reported in the results is merely used as an example to show the methodology, e.g. ignoring confounding, the result should not be interpreted as scientific research data.

KEYWORDS

anemia, clinical outcome, length of hospital stay, mediation analysis, patient blood management, RBC transfusions

1 | INTRODUCTION

Receipt of a peri-operative red blood cell (RBC) transfusion is associated with worse clinical outcomes in patients compared to those who do not receive an RBC transfusion.[1–5] In addition, anemia in pre-operative patients is associated with a higher post-operative complication rate and increased hospital length of stay (LOS) compared to those without anemia.[6–10] In this setting, it may be difficult to disentangle the effect of pre-operative anemia (in general: the exposure variable) from the effect of a peri-operative RBC transfusion itself on the postoperative clinical outcome (in general: the outcome variable). Performing the specific statistical analyses necessary to adequately describe these associations can be complicated. In current literature, there are many ways researchers analyze these relationships. Some papers described non-optimal or even incorrect methods, such as performing a subgroup analysis for patients receiving a peri-operative RBC transfusion or excluding the patients being transfused.[11–13] This leads to information bias, as well as violating the statistical dogma of not conditioning on the future.[14,15] Other studies applied regression analysis, in which RBC transfusions are incorrectly regarded as a confounding variable.[16–18] This paper focuses on the appropriate statistical methodology needed to disentangle the effect of RBC transfusions from the effect of pre-operative anemia on clinical outcome.

2 | CONFOUNDING

Confounders are external variables that can influence the effect of an exposure on the measured outcome. Firstly, confounders need to be related to both the exposure and outcome variables. Secondly, a confounder is not in the causal path between the exposure and the outcome, so the time of occurrence should precede or occur simultaneously with the exposure variable. In clinical studies, confounders can be identified and corrected for in regression analyses. In randomized studies, part of the confounding may have already been compensated for in the randomization process by a more or less equal distribution of the patients' characteristics over the randomized groups.

3 | MEDIATION

A mediator is also an external variable that influences the effect of an exposure on the measured outcome, but it differs from a confounder in two ways. In contrast to a confounding variable, a mediator is situated in the causal path between the exposure and the outcome, meaning that the mediator is affected by the exposure variable. Secondly, confounders and mediators occur at different timepoints toward the outcome measurement. Confounders occur before or simultaneously with the exposure variable, while a mediator occurs afterwards.

Some variables cannot be considered a mediator, regardless of the situation, as they cannot be in the interior of a causal path; for example, sex, since sex cannot be affected by any exposure.

Sometimes, mediation is confused with moderation (also known as effect modification). A variable is a moderator when patients in groups based on the moderator experience different effects of the exposure.[19] A moderator is not situated in the causal path. For example, sex can be viewed as a moderator in many studies, since men and women often have different outcome frequencies when given a certain treatment. In this paper, we did not investigate moderation.

3.1 | A problem "confounder" adjustment cannot fix

As described earlier, confounders and mediators are both related to the exposure and the outcome, but they differ regarding the causal path. An example in transfusion medicine research is further outlined here: when analyzing the effect of pre-operative anemia (exposure) in orthopedic patients undergoing hip- or knee replacement surgery on post-operative outcome (for example length of hospital stay), it is important to investigate the effect of intra- and post-operative RBC transfusions (possible confounder/mediator), assuming that all RBC transfusions occurred after the pre-operative anemia. To decide how to investigate the role of RBC transfusions, we need to look at the causal path and at the timeline of events. To

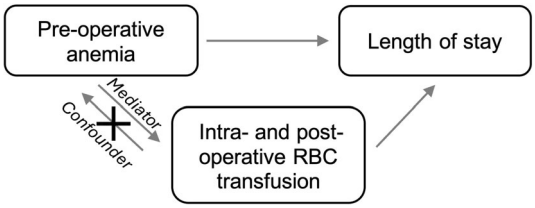

FIGURE 1 Causal pathways of intra- and post-operative RBC transfusion as mediator compared to pre-operative anemia (exposure) and length of stay (outcome). RBC transfusions cannot be regarded as a confounder (see the crossed arrow in the figure), since intra- and post-operative RBC transfusion is a result of the anemia and therefore does not meet the definition of a confounder.

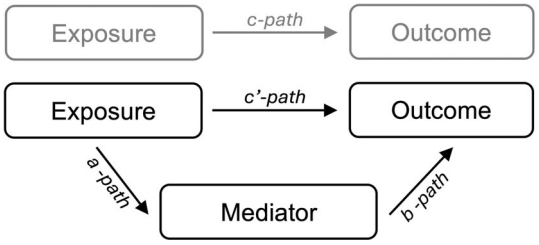

FIGURE 2 Directed acyclic graph (DAG) of the association of an exposure and outcome and the causal pathway of the mediator, arrows directing in the same direction.

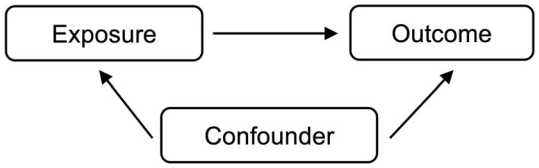

FIGURE 3 Directed acyclic graph (DAG) of a non-causal pathway in the case of a confounder in the association between an exposure and outcome, arrows directing in opposite directions.

meet the definition of a confounder, an RBC transfusion would have to influence whether someone has anemia and whether someone suffers from a complication. Additionally, its occurrence in time should be clarified; in order to comply with the principles of a confounder, the RBC transfusion should occur before or during the pre-operative anemia. While an RBC transfusion does seem to influence complications, the RBC transfusion does not influence anemia, since anemia was present before the RBC transfusion was administered. This means that the RBC transfusion cannot be considered a confounder. Therefore, RBC transfusions should not be corrected for according to the confounder methodology. This problem can be further explored by analyzing the RBC transfusion as a mediator. In this case, the RBC transfusion is placed in the timeline after the exposure variable anemia as it is situated in the causal path. Furthermore, the more severe the anemia, the more RBC units may be transfused. Figure 1 illustrates the causal path of the abovementioned variables in this situation. It should be noted that variables can vary in being labeled as confounder or mediator, depending on the patient population and outcome measures under investigation.

3.2 | Analysis of RBC use as a mediator

In order to identify RBC use as a mediator, the mediator pathways need to be identified. A basic traditional mediation approach consists of four pathways, measured using regression analysis. These pathways are displayed in Figure 2 and will be explained here. Firstly, the association between the exposure (anemia) and the outcome (LOS) is analyzed. This association is called the total effect of the exposure on the outcome, also known as the c-path (see Figure 2). The following step is to add the mediator variable to the analysis. The association between the exposure and the outcome, taking into account the mediator variable, is called the direct effect (also known as the c'-path; see Figure 2). The difference between the total effect and the direct effect is known as the indirect effect of the exposure on the outcome (i.e. the effect going through the mediator). This method to analyze mediation is known as the change in coefficients method.[20] Another method often used to analyze mediation is the product of coefficients method. In this method, first the association between the exposure variable (anemia) and the mediator (RBC) is analyzed. This association is known as the a-path (see Figure 3). Secondly, the association between the mediator (RBC) and the outcome variable (LOS) is analyzed. When this association is analyzed, taking into account the exposure variable, this association is known as the b-path. To calculate the indirect effect size of the exposure (anemia) on the outcome (LOS), through the mediator, the a-path and b-path are multiplied with each other.[21,22] Both methods lead to the same results, as shown in the example below.

A commonly used way to test for significant mediation is the test of joint significance. When both the a- and b-paths are statistically significant, the test of joint significance is positive, this supports the hypothesis that the variable is a mediator.[21,23] Another way to test mediation for significance, is the Sobel formula which can be used to calculate the standard error of the mediation and thus also a 95% confidence interval. Bootstrapping is another frequently used method of calculating the 95% confidence interval for the mediation.[24]

Table 1 shows a summary of the different paths in traditional mediation analysis with their alternative names.

TABLE 1 Summary of mediation pathways, alternative names, and explanations.

Path	Name	Explanation
c-path	Total effect	Association of exposure and outcome
c'-path	Direct effect	Association of exposure and outcome, with a mediator added to the model
a-path	–	Association of exposure and mediator
b-path	–	Association of mediator and outcome with the exposure added to the model
a-path*b-path c-path – c'-path	Indirect effect	Association of exposure and outcome through the mediator

There are several conditions and prerequisites a mediator needs to comply with, which are outlined by Fairchild and McDaniel.[17] Ideally, variables for mediation analysis should be collected in a longitudinal study within a reasonable timeframe in relation to each other, to correctly place the variables on the causal path.[25] On the other hand, mediation analysis has also been performed in cross-sectional studies, based on the biological plausibility of the mediator variables.

TABLE 2 Patient characteristics extracted from the clinical study database.[21,22]

Total population, *N*	**2442**
Length of stay	
Mean (SD)	7.66 (5.56)
Median (IQR)	6 (5–8)
Pre-operative anemia, *N* (%)	274 (11.2)
Peri-operative RBC transfusion, *N* (%)	266 (10.8)

3.3 | An example

In current literature, peri-operative RBC transfusions are often wrongly analyzed as confounders to study the association between peri-operative exposures, e.g., anemia, and post-operative outcomes.[16–18] Not exploring the possibility of mediation may yield misleading results. To illustrate the methodology of mediation analysis, we used data from a previous study, consisting of 2442 patients undergoing elective hip or knee arthroplasty.[26,27] In this study, anemia is calculated from hemoglobin (Hb) levels according to the WHO definition, using a cut-off for men of 13 g/dL and for women of 12 g/dL.[28] For this example, the effect of pre-operative anemia (from now on denoted as anemia) as the exposure on LOS as continuous outcome is analyzed. The number of intra- and post-operative RBC transfusions (from now on denoted as RBC transfusions) were scored up to 14 days as a continuous variable. LOS was defined as the time between the day before surgery and hospital discharge. For this analysis, we assumed that all RBC transfusions occurred *after* the anemia, and for the simplicity of the model, we excluded possible confounders. In Table 2, data are shown on which this example is based.

The analyses were performed using linear regression analyses in IBM SPSS statistics version 28.0.1.1 (supplement 1: syntax). To calculate 95% confidence intervals for the mediation, the PROCESS macro in SPSS was used, which uses bootstrapping to estimate the intervals. Another possibility is to calculate the standard error using the Sobel formula.[29] A problem, however, is that there is no reliable way to calculate statistical significance for the indirect effect. This is due to the phenomenon that the standard error or confidence intervals of an indirect effect are dependent on the strength of the association represented by the a-path and b-path. This is a drawback of this methodology. However, focusing more on the clinical relevance of a mediation outcome than on the statistical significance is also of importance.[20]

When analyzing the association of anemia and LOS, a regression coefficient of 1.48 is found with a 95% CI of [0.78–2.17], $p < .001$, suggesting that patients with anemia have a statistically significant longer LOS by 1.48 days than patients without anemia (total effect). Adding RBC transfusions as a mediator to the model, a regression coefficient for anemia of 0.50 is found with a 95% CI of [−0.17–1.16], $p = .144$. Now, the indirect effect of the mediator can be calculated using the difference in coefficients method. With this method, the direct effect is subtracted from the indirect effect (c-path minus c'-path). In this example, the indirect effect is therefore 1.48–0.50 = 0.98.

The indirect effect can also be calculated using the product of coefficients method. With this method, the regression coefficients of the a-path and b-path are multiplied with each other. The a-path (association between anemia and RBC transfusion) resulted in a regression coefficient of 0.50, $p < .001$ (95% CI 0.38–0.62), meaning that patients with anemia received 0.50 more RBC transfusions than patients without anemia. The b-path (association between RBC transfusions and LOS

TABLE 3 Mediation model results.

	Regression coefficient	*p*	95% CI
Total effect of anemia on LOS (c-path)	1.48	<.001	0.78–2.17
Direct effect of anemia on LOS through RBC transfusion (c'-path)	0.50	.144	−0.17 to 1.16
Association of anemia and RBC transfusion (a-path)	0.50	<.001	0.38–0.62
Association of RBC transfusion and LOS (b-path)	1.96	<.001	1.75–2.17
Association of anemia and LOS through RBC transfusion (indirect effect, a*b-path = c – c')	0.98	–	0.62–1.38

taking into account anemia) resulted in a regression coefficient of 1.96, $p < .001$ (95% CI 1.75–2.17), concluding that if a patient received one extra unit RBCs, LOS increased by 1.96 days. When multiplying the a-path and b-path, the same indirect effect of 0.98 is found.

We calculated the 95% confidence interval around the indirect effect by using bootstrapping and found an interval of [0.62–1.38]. In mediation analysis, sometimes, the proportion mediated is calculated. The proportion mediated is a measure which indicates how much of the total intervention effect goes through a particular mediator. The proportion mediated is calculated by dividing the indirect effect by the total effect, which is in this example 0.90 / 1.48 = 66%, meaning that 66% of the effect of anemia on LOS goes through RBC transfusions. It should be remembered that these numbers are not based on a complete analysis, since confounders are not included, but merely as an example to show the pathways and how mediation analysis is being performed. Therefore, these numbers cannot be interpreted in the light of a full data analysis.

Table 3 provides an overview of the results of the different analyses performed.

3.4 | Mediation analysis in transfusion medicine literature

The methodology of mediation analysis has not been reported much in transfusion literature. We identified three papers which incorporated this methodology using RBC transfusions as mediators in the regression model.[30–32] All three papers were performed in surgery patients (two studies in cardiac surgery, one in non-cardiac surgery) and used large databases. These papers were the first in transfusion medicine literature analyzing the association between preoperative anemia and clinical outcomes through RBC transfusions as mediators. Interpretation of the results can still be difficult, since there may be residual confounding or non-reported confounding. Furthermore, incomplete and/or inconsistent reporting of the mediation analyses was found. These papers, however, show an adequate blueprint of how to incorporate mediation analysis into transfusion medicine research.

The impact of RBC transfusions on clinical outcome is important since several studies promote transfusion alternatives to avoid RBC transfusions.[33,34] In some of the recently published papers, where mediation analysis has been used to disentangle the role of RBC transfusions in anemic patients undergoing surgery, the authors concluded that clinical outcome was highly mediated by RBC transfusions, meaning that RBC transfusions worsen clinical outcome.[30,31] If this is true, RBC transfusions should indeed be avoided. On the other hand, if it turns out that, following mediation analysis, the effect of RBC transfusions is small, it may suggest that replacing or reducing RBC transfusions by other Patient Blood management (PBM) interventions may not improve clinical outcome.

4 | DISCUSSION

We outlined the use of traditional mediation analysis in transfusion medicine, providing the basic rules how to identify a variable as mediator, provided an example to show the pathways, and how to analyze this using linear regression analyses with data from an existing database. It is of vital importance that a structured approach is followed in identifying and analyzing a mediator and on consistency in reporting the results. This should include reporting of a figure that clearly shows the causal pathways, which can be performed, by the use of a directed acyclic graph (DAG), a figure to illustrate the causal or non-causal paths in a model. Figures 2 and 3 are examples of a very simple DAG. The arrows display the direction of the effect that variables have on each other. As described by Digitale et al.: "Paths are causal if each variable causes the subsequent variable (all the arrows point in the same direction)".[35] The DAG with the mediator in Figure 2 shows an example of a causal path. The DAG with a confounder in Figure 3 shows a non-causal path. It should be noted that although a DAG gives an adequate overview of the relation between the

variables, it lacks a timeline of the variables. It is important to carefully choose the right timepoints to measure the variables at appropriate intervals from each other to ensure relevant causality.[25] The results of our example can by all means not be used as real valid data and no consequences should be drawn on the results, since the analysis is explicitly performed to only show the methodology. When performing data-analysis, one should take into account all possible confounders between exposure and mediator and/or between mediator and outcome, which for the sake of simplicity, we now left out. Our simplified model only included the three variables needed for a basic mediation analysis: pre-operative anemia (exposure), LOS (outcome) and peri-operative RBC transfusion (mediator). We also assumed a causal relationship and that all RBC transfusions were administered after pre-operative anemia occurred. In practice, it is not always possible to obtain information on the specific time points of the occurrence of certain events such as RBC transfusions or the occurrence of an adverse post-operative outcome, for example an infection.

There are different ways to report the results from mediation analysis, depending on the type of mediator and outcome. A mediator can either be continuous or dichotomous. This influences both the type of regression analysis needed to calculate the separate paths and the necessary procedure to calculate the indirect effect. For a continuous mediator and outcome, traditional mediation analysis as described earlier can be used. When the outcome variable is dichotomous (for example infection complication) and the mediator is continuous, the product-of-coefficients method cannot be used. This is due to the fact that for the a-path, a linear regression analysis has to be used, while for the b-path, a logistic regression analysis has to be used. The difference in coefficients method, however, can always be used.

For a linear mediator (for example the number of RBC transfusions) with a linear outcome (for example LOS), the proportion mediated is a properly validated reporting measure. It represents the relative effect of the mediator on the association between the exposure and the outcome.[36,37] We calculated the proportion mediated by dividing the effect of anemia on complications by RBC transfusion as mediator (indirect effect, a*b-path) by the effect of anemia on complications without RBC transfusion as mediator (total effect, c-path). In our example, the proportion mediated was 66%.

In some situations, traditional mediation analysis as described in this paper is not the most appropriate. When the mediator and/or outcome are not continuous, it is suggested to use another method, named causal mediation analysis.[20]

In conclusion, the use of mediation analysis is a valid statistical method in patient blood management studies or studies evaluating the role of RBC transfusions on clinical outcomes. The first important step is to identify the RBC transfusion variable as a potential mediator and subsequently identify the causal pathways and timeline of the variables under investigation to determine how such variables can be properly analyzed. Interpreting a mediator as if it is a confounder leads to incorrect results. It is of critical importance to know whether RBC transfusions may influence clinical outcomes in order to predict whether PBM measures, resulting in a decrease of RBC transfusions, are successful and will improve clinical outcomes.

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CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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