International Blood Research & Reviews

Volume 15, Issue 3, Page 39-52, 2024; Article no.IBRR.125550
ISSN: 2321–7219

Using Soluble CD38 to Overcome Daratumumab Interference in Pretransfusion Compatibility Testing

Michela PIVETTA [a] and Gianluca GESSONI [a,b++*]

[a] Transfusion Medicine Dell'Angelo General Hospital Mestre (Venice), Italy.
[b] Transfusion Medicine Department of Venice Prefecture, Dell'Angelo General Hospital, Via Paccagnella 11, 30172 Mestre (Venice) Italy.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ibrr/2024/v15i3343

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125550

Original Research Article

Received: 26/08/2024
Accepted: 28/10/2024
Published: 04/11/2024

ABSTRACT

Background: CD38 is a protein highly expressed on myeloma (MM) cells that has been shown to be an effective target antigen for monoclonal antibody therapies, so treatment with anti-CD38 monoclonal antibodies is a first line therapy for patients with MM. Although CD38 is weaker expressed on erythocytes anti-CD38 binds to CD38 on reagent RBCs and cause panreactivity *in vitro* and subsequent false positive reactions in indirect antiglobulin tests (IAT), antibody detection (screening) tests, antibody identification panels, and anti-human globulin (AHG) crossmatches. These findings suggest that the soluble CD38 method could improve transfusion safety for patients receiving anti-CD38 therapy, particularly in urgent clinical settings This study aims to evaluate the effectiveness of a new soluble CD38-based method in mitigating Daratumumab interference during pretransfusion compatibility testing."

[++] Director;
**Corresponding author: E-mail: gianluca.gessoni@aulss3.veneto.it;*

Cite as: *PIVETTA, Michela, and Gianluca GESSONI. 2024. "Using Soluble CD38 to Overcome Daratumumab Interference in Pretransfusion Compatibility Testing". International Blood Research & Reviews 15 (3):39-52. https://doi.org/10.9734/ibrr/2024/v15i3343.*

Methods: We evaluated the Grifols sCD38 method in 20 patients and compared it with the DTT method

Results: In our experience the sCD38 method reduced testing time from 150 to 50 minutes and demonstrated 100% efficacy, compared to 90% with DTT. The sCD38 effectively mitigated the interference caused by anti-CD38 antibodies in 10 (100% efficacy) of patient samples tested while DTT was successful in only 9/10 (90% efficacy); no interference was observed in patients presenting anti erythrocyte antibodies. Moreover, there was no negative influence on DTT sensitive blood group systems such as KEL upon sCD38 treatment.

Conclusions: In our Laboratory, in the year 2023, we processed 129 patients treated with anti-CD38. Therefore the reduction from 150 to 50 minutes in the time needed to perform tests for mitigation of anti-CD38 interference appears to be relevant with a recovery of approximately 210 technical-hours per year. Another highly appreciated operational aspect was the possibility of treating the patient's plasma and perform tests using automatic instruments (Erytra Grifols) available in our laboratory. In the evaluation of this new method, we did not observe failures in the mitigation of anti-CD38 interference. Furthermore, the results obtained in the samples that presented allo or auto antibodies were not affected by the treatment with sCD38. In our experience Grifols sCD38 assay is straightforward and quick to perform ant it is superior to DTT treatment in the mitigation of anti-CD38 antibody interference in MM patients treated with Daratumumab.

Keywords: CD38; daratumumab; interference; mitigation; pretransfusion tests; soluble CD38.

1. INTRODUCTION

The CD38 antigen (cyclic ADP ribose hydrolase) is a transmembrane glycoprotein (PM approximately 31 kDa) that appears to be involved in three different cellular functions: adhesion protein, signal transduction, regulation of the calcium signalling pathway; and it is encoded by the *CD38* gene located on chromosome 4 (Leleu et al., 2022, De Novellis et a.., 2022). The CD38 antigen is present on the surface of numerous cell lines such as B, T and NK lymphocytes, plasma cells but also on platelets and erythrocytes. CD38 is particularly expressed in neoplastic plasma cells of multiple myeloma (MM) (Gozzettiet al., 2022, Shenet al., 2022). Anti-CD38 monoclonal antibodies (MoAb) have a good therapeutic effect in patients with MM by inhibiting the growth and proliferation of neoplastic plasma cells both through direct mechanisms such as enzymatic inhibition and induction of apoptosis, and through immune-mediated mechanisms such as antibody-dependent or complement-dependent cellular toxicity (Offidani et al., 2021, Yanq et al., 2023).

The fact that CD38 is expressed on the surface of red blood cells implies a binding of anti-CD38 MoAb to erythrocytes, which will then be recognized and removed from the bloodstream at the level of the splenic reticuloendothelial system, mediated by binding to the Fc receptor. This extra-vascular haemolysis can lead to anaemia that in 25-30% of subjects will be of such an extent as to require transfusion support (Mei et al., 2019, Nedumcheril et al., 2021). On the other hand, the presence of anti-CD38 MoAb on the surface of mature erythrocytes is the basis of the interferences observed in the Immunohematology Laboratory (Mei and Wool, 2019, Nedumcheril et al., 2021).

Currently, different anti-CD38 MoAbs with different characteristics are available or in an advanced stage of experimentation: Daratumumab (human IgG1-k), Isatuximab (chimeric IgG1-k), Felzartamab (IgG1-L), Mezagitamab (IgG1-L), which are directed towards different epitopes of the CD38 antigen, and which present different percentages of interference with pre-transfusion tests: from 100% observed for Daratumumab to 65% reported for Isatuximab. This interference, in the case of Daratumumab (DARA), can be detected up to 3-6 months after the last administration of the drug (Chami et al., 2022, Agrawal et al., 2020).

In Immunohematology Laboratory, DARA interference with serological tests occurs in all methods that use Coombs serum (antiglobulin serum) while usually the auto control test (patients' plasma with patients' RBCs in antihuman immunoglobulin) is negative because CD38 expression is downregulated during treatment therefore no haemolysis is observed *In vivo* (Sullivan et al., 2017, Chapuy et al., 2015). Table 1 lists the main immunohematology tests with the possibility of interference from DARA.

To mitigate the interference of DARA in alloantibody screening and pretransfusion testing various methods have been proposed. For example, methods for the detection of anti-erythrocyte antibodies have been proposed that do not involve the use of antiglobulin serum, such as tests in solid phase tube tests using potentiating agents such as polybrene; however, these methods have often demonstrated a less than fully satisfactory sensitivity in the detection of clinically significant antibodies (Perram et al., 2020, Zhou et al., 2021, Feng et al., 2023). Alternatively, methods have been proposed that involve the use of proteolytic enzyme-treated enzyme panels, however, enzyme treatment destroys some erythrocyte blood group antigens (i.e. M,N Lea and Leb) (Carreño-Tarragona et al., 2019). Treatment of test cells with dithiothreitol (DTT) is widely used in serological laboratories. DTT denatures CD38 on the cell surface by reducing disulfide bonds. However, DTT also destroys or modifies some other blood group antigens, e.g., KEL, DO, JMH, LU, IN, and YT, which results in impaired sensitivity to detect alloantibodies against these blood group antigens (Chapuy et al., 2016, Furumaki et al., 2021, Gessoni et al., 2020, Hosokawa et al., 2018). Recently has been suggested a method to overcome DARA interference by blocking the CD38 epitopes on RBCs by DARA-Fab fragments which were generated by papain proteolysis of DARA antibodies (Habicht et al., 2023). Blocking of the antigen binding site of DARA by incubation of patients' plasma with anti-idiotypic antibodies was also suggested and may also be intriguing; however, these antibodies are not commercially available. In addition, this assay requires umbilical cord RBCs as screening cells that are not typically available in a routine transfusion laboratory, and, furthermore, cord cells may have altered expression of some antigens (Aung et al., 2022). An additional alternative to mitigate Daratumumab interference on pre-transfusion testing is to treat the patient's serum with soluble CD38 peptides with the intent of blocking the DARA binding site (Bindaet al., 2018, National Health Service UK). See Table 2 for a quick overview of the methods that can be used to mitigate interference from anti-CD38 on pre-transfusion tests.

Aim of the study: In this paper we report a comparative evaluation of two methods for mitigation of anti-CD38 interference on pre-transfusion tests. As matter of facts we compared the in-house method, based on the pretreatment of red blood cells with Dithiothreitol (DTT) 0.2M, with a commercial method based on the pretreatment of patient plasma with soluble CD38 antigen (sCD38).

2. MATERIALS AND METHODS

The Immunohematology Laboratory of Transfusion Medicine in Mestre is located in Dell'Angelo a large General Hospital that acts as a provincial hub for a network of ten Spoke Hospitals in an area of about one million people. Our Transfusion Medicine has implemented a second-level immunohematology laboratory that has routine access to three different platforms for performing serological tests: test tubes, using a semi-automated in-house method; test cards, using a commercial automated method (Grifols); solid-phase test, using a commercial automated method (Werfen). For genotyping we adopted a commercial automated method (Bead-Chip, Werfen). Our extended phenotyping protocol includes the study of the following erythrocyte blood group antigens: ABO, C, E, c, e, K, k, Kpa,b, Fya,b, Jka,b, M, N, S, s, Lua,b, P1, Xga, Lea,b, Our extended genotyping protocol includes the study of the genes coding for the following erythrocyte blood group antigens: C, E, c, e, K, k, Kpa,b, Jsa,b, Fya,b, Jka,b, M, N, S, s, Lua,b, Dia,b, Doa,b, Hy, Joa, Coa,b, Sc1,2, LWa,b. The Laboratory also can search for anti-platelet antibodies and anti-HLA antibodies using a Luminex analyser. In our Provincial Department of Transfusion Medicine (DIMT) second level immunohematology assays, including the management of patients treated with anti-CD38, have been centralized in the Mestre Laboratory, from August 2023 to July 2024 our Laboratory processed 129 transfusion requests for patients treated with anti-CD38, all related to patients with multiple myeloma treated with Daratumumab.

Mitigation of interference from anti-CD38 with DTT method: To mitigate interference from Daratumumab in our Laboratory we use a gel card method with pretreatment of red blood cells with 0.2M dithiothreitol (DDT) which can denature the CD38 antigen present on the surface of the red blood cells, minimizing the interference of the drug on immunohematology tests. To prepare the 0.2M DTT solution, dissolve 1 gram of DTT in 32 mL of PBS buffer or saline. Once prepared, the solutions can be aliquoted and stored for up to 5 days at +4°C, or frozen at -30°C; the aliquot of the DTT solution must be brought to room temperature and shaken gently before use. The red blood cells treated with DTT

can be stored for up to 5 days at +4°C if resuspended in PBS or saline. Table 3 provides a brief description of the method. When interpreting the results, it must be kept in mind that treatment with DTT can destroy some erythrocyte blood group antigens (Kell, Penney, Sutter, Dombrock, Lutheran and others), thus making some antibody specificities undetectable (Chapuy et al., 2016, Furumaki et al., 2021, Gessoni et al., 2020, Hosokawa et al., 2018).

Mitigation of anti-CD38 interference with sCD38 method: The test uses a protein reagent composed of a recombinant human CD38 protein (extracellular domain) in soluble form that, once mixed with the serum of the patient treated with Anti-CD38, binds to the monoclonal antibody neutralizing it, thus eliminating/reducing its interference on immunohematology tests such us the indirect Coombs test. The use of the sCD38 reagent does not remove the positivity of the indirect Coombs test due to the presence of alloantibodies, even if the reactivity of the anti-Fya and anti-Fyb antibodies may be decreased. All the necessary reagents are ready for use (Binda et al., 2018, National Health Service UK). Table 3 shows a schematic comparison between the 0.2M DTT method routinely used in our laboratory and the sCD38 method.

Samples: As reported in Table 4 for comparison of DTT and sCD38 methods efficiency in mitigation of DARA interference on pretransfusion tests we selected twenty samples: ten obtained from patients in treatment with DARA and ten obtained from patient not in therapy with DARA. In the second group five patients had a positive IAT (anti-E, anti-D, anti-K, anti-c, anti-Fya); in the first group four patients had a positive IAT (anti-c, anti Jka, anti-Fya, anti-M).

To study stability of sCD38 mitigation we performed a IAT in three samples obtained from patients in therapy with DARA for a MM, before sCD38 treatment, immediately after sCD38 treatment and aftermath every 24 hours for a week (storage performed at +4°C).

Pretransfusion tests: To perform the evaluations reported in this study, the methods currently in routine use at our Laboratory were used. For the search for anti-erythrocyte antibodies, we used a Liss-Coombs micro column method, testing the patient's serum against a three-cell screening panel. For each patient, two units of theorically compatible packed red blood cells were cross-matched using a Liss-Coombs microcolumn method (Gesson, 2024).

3. RESULTS

This study focuses on the comparison of two methods designed to mitigate the interference of DARA on pre-transfusion tests. The in-house method using DTT 0.2M historically used in our laboratory was therefore compared with a commercial method based on the use of sCD38**.**

Patients non treated with DARA: In samples obtained from the ten subjects not in therapy with DARA no interference was detected in IAT. Five samples without anti-erythrocyte alloantibodies showed a negative IAT using native plasma tested against untreated or DTT-treated red cell panels; the sCD38-treated plasma was also non-reactive when tested against standard red cell panels. Five samples with anti-erythrocyte alloantibodies showed a positive IAT using native plasma tested against untreated or DTT-treated red cell panels; the sCD38-treated plasma was also reactive when tested against standard red cell panels.

Patients treated with DARA: Considering ten MM patients treated with DARA, all presented a positive IAT when immunohematology assays were performed without methods to mitigate interference from anti-CD38. In six samples without anti-erythrocyte alloantibodies, DTT treatment eliminated anti-CD38 interference in five (83%) of them, while sCD38 treatment was effective in six patients (100%). The four patients with anti-erythrocyte alloantibodies remained IAT positive using both the DTT and sCD38 methods. These data were reported in Table 4.

Methods' comparison: In Fig. 1 we reported the images of cards relating to the pre-transfusion tests: research on erythrocyte antibodies by IAT (three micro-columns on the left) and crossmatch in Liss-Coombs (two micro-columns on the right) in three patients treated with DARA without alloantibodies. As can be observed, the three samples showed a marked positivity when tested without using methods to mitigate DARA interference. Using the 0.2M DTT method for the treatment of the red blood cells before performing the IAT and the crossmatch, we obtained a marked mitigation of the interference from DARA. However, the microcolumns were never frankly negative, thus requiring manual interpretation by the operator. Furthermore, in the third sample, one of the two crossmatches was positive. Using the sCD38 method to treat the patient's plasma before performing the IAT and crossmatch, we observed a complete

elimination of DARA interference. Red blood cells had precipitated forming a compact layer on the bottom of the micro columns while the overlying phase appeared transparent making it possible to read the cards in complete automation.

Duration of Mitigation: To evaluate the duration of the mitigation of interference from Daratumumab on pre-transfusion tests, we performed on selected patient on DARA therapy, we performed an IAT before the treatment with sCD38 and then for a week every 24 hours. As reported in Fig. 2, the IAT performed on untreated plasma showed a marked positivity from interference that was eliminated after treatment with sCD38 with a mitigation that remained constant for seven days.

Time efficiency: The average execution time of the DTT method in our Laboratory was 150 minutes versus 50 minutes for the sCD38 method as reported on Table 3.

4. DISCUSSION

In patients with Multiple Myeloma, the frequency of occurrence of anti-erythrocyte alloantibodies is rather low (around 3%). This reduced incidence of alloimmunizations can be attributed to immune depression due both to the disease itself and to its treatment (Oostendorp et al., 2015, Bullock et al., 2021, Zhan et al., 2020). However, despite these low levels of alloimmunization, considering the difficulties in identifying and characterizing any anti-erythrocyte alloantibodies, the SIMTI (Italian Society of Transfusion Medicine and Immunohematology) guidelines recommend that patients with Multiple Myeloma, before starting therapy with DARA, undergo at least typing for ABO, Rh, Kell, TCD and TCI. Extended serotyping and/or genotyping are also recommended (Matteocci et al., 2023). We have therefore agreed with the Oncology and Hematology Departments of the Province of Venice that in all patients who are candidates for anti-CD38 therapy, before starting the treatment, a whole blood sample in EDTA will be taken which will be sent to our Immunohematology Laboratory for the execution of extended serotyping and genotyping (Coluzzi et al., 2018). If the patient comes to our attention after having already started therapy with anti-CD38, our protocol does not include the execution of extended phenotyping but only genotyping (Matteocci et al., 2023, Coluzziet al., 2018).

At our Transfusion Medicine Department (DIMT), we perform extensive phenotyping and genotyping of regular donors of group O and A, selected based on the presence of particular Rh and Kk phenotypes, using high-throughput automated systems. So we have a database, with several thousand extensively typed regular donors that allows to select units of packed red blood cells with level III compatibility with the patient candidate for transfusion therapy (Matteocci et al., 2023, Coluzziet al., 2018).

Our protocol for assigning red blood cell concentrates in patients with MM treated with anti-CD38 is extremely prudent and includes both the search for irregular antibodies by IAT and the execution of the crossmatch (gel-card method with Liss-Coombs). All transfusion requests of patients treated with anti-CD38 and the related samples, are transferred to Mestre. In our immunohematology laboratory, the routine method for mitigation of DARA interference is pre-treatment with DTT (0.2M) both for the polyantigenic group zero test red blood cells used for antibody screening and for the red blood cells of the units to be transfused. This is an internationally validated method that has been validated in-house at our Laboratory (Chapuy et al., 2016, Furumaki et al., 2021, Gessoni et al., 2020, Hosokawa et al., 2018). This method is quite complex and requires trained and expert personnel for its execution. Furthermore, in our operational experience, it requires on average 150 minutes for the result and therefore is not suitable for managing urgent requests. Even from the point of view of interpretation of the results, trained and expert personnel are necessary since, as reported in Fig. 1, treatment with DTT, although mitigating the interference, will never give a complete negativization of the pre-transfusion tests. If the card shown in the figure is read automatically, the expert system for interpreting the results will give a weak positivity (+---) for TCI and an incompatibility for crossmatch. It will therefore be necessary, to proceed with a manual validation of the results. We therefore welcomed the possibility of adopting a method such as the one based on the mitigation with sCD38 of the interference from anti-CD38 on pre-transfusion tests which, although more expensive, promised to be quicker and easier to perform (Del Bello et al., 2023, Anani et al., 2017). Also in this case, it is a method validated at an international level (Lejon et al., 2023, Bise et al., 2023, Engstrom et al., 2023, Legoff et al., 2023) and has been validated

Table 1. Daratumumab interference in Immuno Haematology Assays

Assays description	Interference
ABO typing	**No** interference
RhD typing	**No** interference
Test for D weak	**Yes** presence of Interference
RhCE typing	**No** interference
Kell antigen	**No** interference
k antigen	**Yes** presence of Interference
Autocontrol	**No** interference
Indirect Antiglobulin Test	**Yes** presence of Interference
Direct Antiglobuli Test	**Yes** presence of Interference
Crossmatch	**Variable** depending on the method used
Typing for other erythrocyte blood group antigens	**Variable** depending on the method used

This table schematically reports the routine immune haematological tests and the possibility of interference by treatment with Daratumumab (Sullivanet al., 2017, Chapuyet al., 2015)

Table 2. Laboratory methods to mitigate Daratumumab interference in pretransfusion tests

Mitigation Strategy	Mechanism of action	Advantage	Disadvantages
Solid phase tests without antiglobulin serum (Perram et al., 2020)	DARA interference is reduced by not using antiglobulin serum.	Fast, simple and inexpensive,	sub-optimal sensitivity
Tube tests without antiglobulin serum (Zhou et al., 2021, Feng et al., 2023)	DARA interference is reduced by not using antiglobulin serum.	Fast, simple and inexpensive,	sub-optimal sensitivity
Red blood cells treated with proteolytic enzymes (Carreño-Tarragonaet al., 2019)	Proteolytic enzymes destroy CD38 molecules on the surface of erythrocytes	Fast, simple and inexpensive,	Highly variable efficacy. Treatment with proteolytic enzymes destroys some erythrocyte blood group antigens
Treatment of red blood cells with DTT at various concentrations (0.04M, 0.1M, 0.2M). (Chapuy et al. 2016, Furumaki et al., 2021, Gessoni et al., 2020, Hosokawa et al., 2018)	DTT denatures CD38 present on the surface of red blood cells	inexpensive	Long to perform, requires expert personnel. Destroys some erythrocyte blood group antigens
treatment of the patient's plasma with FAB fragment (Habicht et al., 2023)	Fab fragments bind to CD38 and prevent it from binding to DARA	Commercial test. Simple and fast execution.	Expensive

Mitigation Strategy	Mechanism of action	Advantage	Disadvantages
treatment of the patient's plasma with anti-idiotype antibodies. (Aung et al., 2022)	Anti-idiotype antibodies neutralize DARA in the patient's plasma	Commercial test. Simple and fast execution.	Expensive
treatment of the patient's plasma with soluble CD38 (Binda et al., 2018, National Health Service UK)	il CD38 solubile neutralizza il DARA nel plasma del paziente	Commercial test. Simple and fast execution.	Expensive

This table schematically reports the various strategies that can be used to mitigate the interference of daratumumab on pre-transfusion tests. The first column schematically describes the mitigation strategy with the relative bibliographic references. The second column indicates the mechanism of action, the third column reports the advantages, and the fourth column reports the disadvantages of the proposed method

Table 3. Comparison of methods adopted in our Laboratory to mitigate Daratumumab interference on pre transfusion tests

Routine Method: DTT 0.2M	New methos: Soluble CD38
Before performing the procedure, it is recommended to perform an antibody test (indirect Coombs test) using the plasma of the untreated patient.	Before performing the procedure, it is recommended to perform an antibody test (indirect Coombs test) using the plasma of the untreated patient.
Preparation of 0.2M DTT solution dissolve 1 g of DTT in 32 ml of PBS (pH 7.3). Divide into aliquots to be stored at -30°C.	____
Defrost an aliquot of DTT 0.2M and wait until it reaches room temperature and mix. Allow reagents and samples to reach room temperature before performing the test.	Allow reagents and samples to reach room temperature before performing the test.
Select the test red blood cells to be used as controls: the positive control must be E+ (EE or Ee), the negative control must be K+ (Kk or KK).	____
Appropriately identify the tubes: screening panel cells, donor cells to be crossed, autocontrol, positive control, negative control.	Appropriately identify the tubes: screening panel cells, donor cells to be crossed, autocontrol, positive control, negative control.
Prepare the 3% red blood cell suspensions Red blood cells Text: Commercial panels are made up of a 0.8% red blood cell suspension, therefore the reagents must be prepared manually by centrifuging 500 microliters of solution, eliminating the supernatant and resuspending the bottom in 150 microliters of buffer solution. To prepare the positive and negative control, the self-control and the samples relating to the units to be compatibilized, resuspend 10 microliters of red blood cells in 300 microliters of buffer solution.	The red blood cell test panels are made up of a 0.8% red blood cell suspension and can therefore be used as such. To prepare a 1% suspension of the units to be compatibilized, resuspend 10 microliters of red blood cells in 1000 microliters of buffer solution

Routine Method: DTT 0.2M	**New methos: Soluble CD38**
Wash the red cells of each tube 4 times with PBS buffer pH 7.3 (500 uL PBS for each wash). Add 400 uL (8 drops) of 0.2 M DTT to each tube. Mix well and incubate at 37°C for 30 minutes. During the incubation mix gently by inverting the tubes 3-4 times. Wash the red cells again as described above. Resuspend the washed red cells at 0.8%. The red cells treated with DTT can be stored at + 4°C for up to 5 days.	_____
Label Coombs card wells with cell number or type Inoculate 50 uL of red cell suspension Add 25 uL of patient plasma Incubate for 10 minutes at 37°C Centrifuge in a dedicated card centrifuge Examine each well for hemolysis and agglutination and record the results Interpret the results.	Pretreat plasma with 2 ul sCD38 Grifols for every 25 ul patient plasma to eliminate drug interference. Incubate for 10 minutes at 37°C. Dispense 25 ul pretreated plasma and 50 ul red cell suspension into the card well. Incubate for 10 minutes at 37°C Centrifuge in a dedicated card centrifuge Examine each well for hemolysis and agglutination and record the results Interpret the results.
Average time required to process a sample 150 minutes	**Average time required to process a sample 50 minutes**

Table 4. Samples selected for the comparison study

DARA therapy	**Sample Description**	**IAT before Any treatment**	**IAT after DTT treatment**	**IAT after sCD38 treatment**
NO	Negative	Negative	Negative	Negative
NO	Negative	Negative	Negative	Negative
NO	Negative	Negative	Negative	Negative
NO	Negative	Negative	Negative	Negative
NO	Negative	Negative	Negative	Negative
NO	Positive (anti-E)	Positive	Positive	Positive
NO	Positive (anti-D)	Positive	Positive	Positive
NO	Positive (anti-K)	Positive	Positive	Positive
NO	Positive (anti-c)	Positive	Positive	Positive
NO	Positive (anfi.Fya)	Positive	Positive	Positive
YES	Negative	Positive	Negative	Negative

DARA therapy	Sample Description	IAT before Any treatment	IAT after DTT treatment	IAT after sCD38 treatment
YES	Negative	Positive	Positive	Negative
YES	Negative	Positive	Negative	Negative
YES	Negative	Positive	Negative	Negative
YES	Negative	Positive	Negative	Negative
YES	Negative	Positive	Negative	Negative
YES	Positivo (anti-c)	Positive	Positive	Positive
YES	Positivo (anti-Jka)	Positive	Positive	Positive
YES	Positivo (anti-Fya)	Positive	Positive	Positive
YES	Positivo (anti-M)	Positive	Positive	Positive

IAT: Indirect antiglobulin test

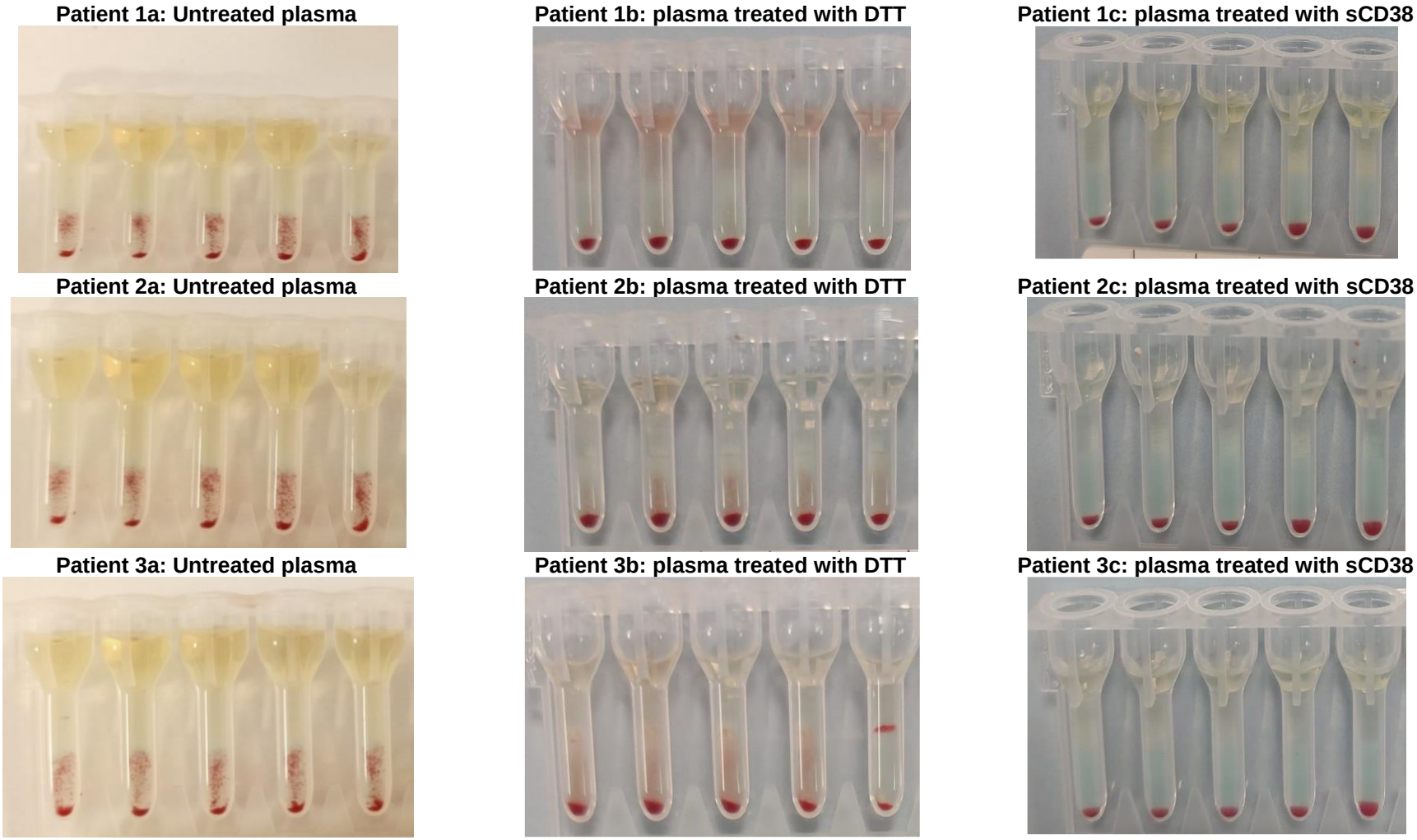

Fig. 1. Comparison of DTT and cSD38 methods for mitigation of Daratumumab interference in pre transfusion tests

For each samples we performed indirect antiglobulin test (three micro-columns on the left) an a cross-match in liss.Coombs (two micro-columns on the right)

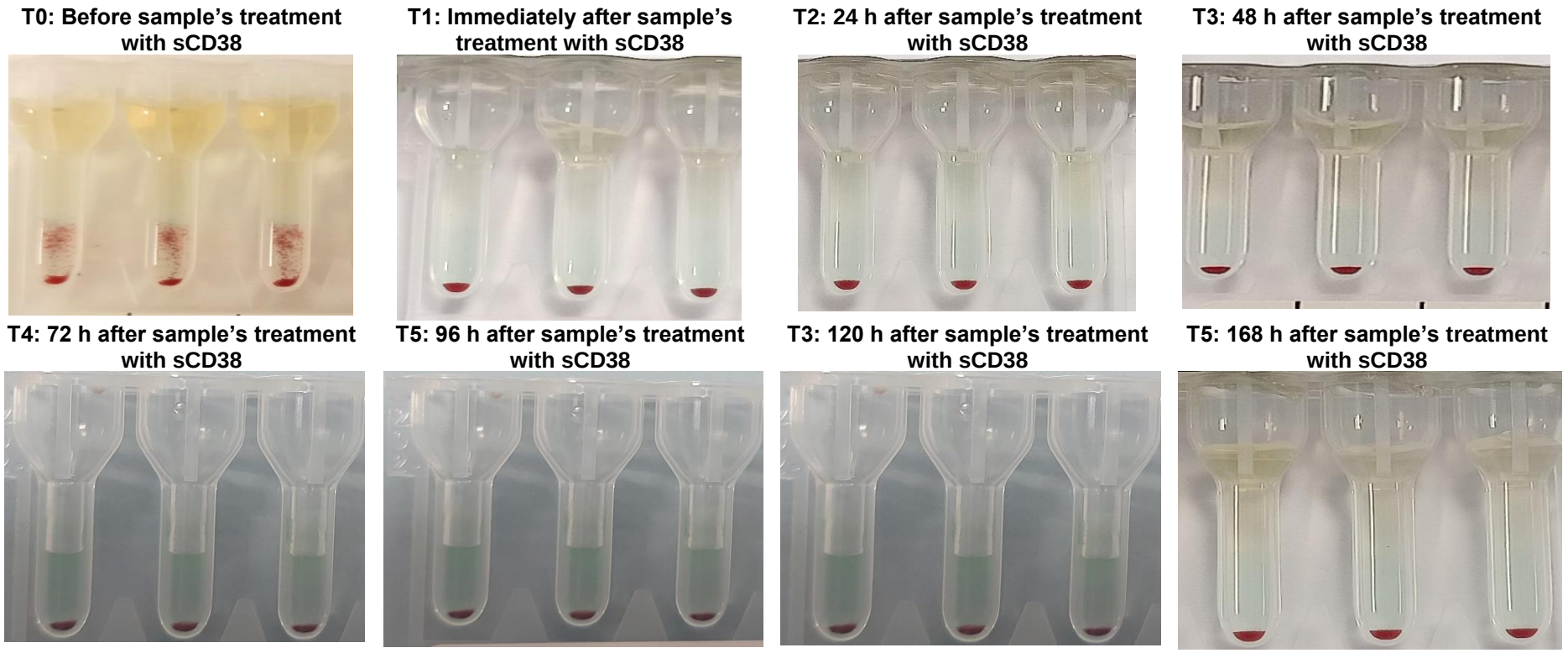

Fig. 2. Mitigation of CD38 interference on pre transfusion test, evaluation of stability of treated plasma until 168 hours

Using a sCD38 soluble based assay for mitigation of Daratumumab interference on pretransfusion test is maintained unchanged for up to seven days

in house at our Laboratory (Pivetta et al., 2024). The main disadvantage of the method based on the use of sCD38 appears to be much more expensive than the methods based on DTT. The main limitation of our study is related to the selection of patients, in fact all were treated with Daratumumab and therefore we could not investigate the efficacy of the sCD38-based method in mitigating interference from other anti-CD38 MoAb used in MM therapy.

5. CONCLUSION

Results obtained in this study have allowed us to highlight how the treatment of the patient's plasma with sCD38 is able to eliminate the interference from DARA on the IAT and on the crossmatches in an effective and complete manner without interfering with the detection of anti-erythrocyte alloantibodies possibly present in the samples. Moreover, the sCD38 based method is relatively simple that involves pre-treatment patients' plasma with a ready-to-use reagent. Plasma treated with sCD38 can be used to perform the necessary pre-transfusion tests using highly automated analysers. The effective mitigation of interference allows the management of results through the middleware in use in our Transfusion Medicine, normally using the pre-set automated validation rules, although manual intervention by operators is always possible,. Plasma treated with sCD38, if stored at +4°C, shows persistent mitigation of DARA interference and pre-transfusion tests can be performed up to seven days after treatment. In our operational experience, the total time from plasma treatment with SCD38 to IAT and crossmatches results is about fifty minutes, therefore, in our opinion, this method is suitable for managing urgent requests (Phou et al., 2021). Retrospectively considering the 129 samples of MM patients treated with DARA that we processed in 12 months, the treatment with sCD38 was completely effective in mitigation of the interference on pre-transfusion tests in 128 cases (99.2%). In this patient we repeated the plasma treatment by doubling the dose of sCD38, opting for the complete mitigation of the interference with complete resolution of the interference itself, presumably due to a high concentration of the drug not neutralized by the standard dosage of ligand.

CONSENT

As per international standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards, written ethical approval has been collected and preserved by the author(s).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT etc.) and text-to-image generators have been used during writing or editing of the present manuscript

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Agrawal, S., Chowdhry, M., Karna, P., et al. (2020). Daratumumab: The perplexity in immunohematology with emerging horizons in myeloma therapy. *Asian Journal of Transfusion Science*, *14*, 200-202. https://doi.org/10.4103/ajts.AJTS_19_19

Anani, W. Q., Marchan, M. G., Bensing, K. M., et al. (2017). Practical approaches and costs for provisioning safe transfusions during anti-CD38 therapy. *Transfusion*, *57*, 1470-1479. https://doi.org/10.1111/trf.14021

Aung, F., Spencer, J., Potter, D., et al. (2022). Efficient neutralization of daratumumab in pretransfusion samples using a novel recombinant monoclonal anti-idiotype antibody. *Transfusion*, *62*, 1511-1518. https://doi.org/10.1111/trf.17006

Binda, M., Favaloro, V., Jody, D., Berry, J. D., & Schwind, P. (2018). Novel soluble CD38 for efficient neutralization of high titer anti-CD38 antibodies. *Transfusion*, *58*(S2), 171A.

Bise, T., Moskwa, C., Selleng, J., et al. (2023). Neutralization of anti-CD38 in patient samples by a soluble CD38 protein to allow alloantibody detection procedures. *56th Annual Meeting German Society for Transfusion Medicine and Immunohematology (DGTI)*, Berlin.

Bullock, T., Foser, A., & Clinkard, B. (2021). Alloimmunisation rate of patients on daratumumab: A retrospective cohort study of patients in England. *Transfusion Medicine*, *31*, 474-480. https://doi.org/10.1111/tme.12808

Carreño-Tarragona, G., Cedena, T., Montejano, L., et al. (2019). Papain-treated panels are a simple method for the identification of alloantibodies in multiple myeloma patients treated with anti-CD38-based therapies. *Transfusion Medicine*, *29*, 193-196. https://doi.org/10.1111/tme.12508

Chami, B., Okuda, M., Moayeri, M., et al. (2022). Anti-CD38 monoclonal antibody interference with blood compatibility testing: Differentiating isatuximab and daratumumab via functional epitope mapping. *Transfusion*, *62*, 2334-2348. https://doi.org/10.1111/trf.17137

Chapuy, C. I., Aguad, M. D., Nicholson, R. T., et al. (2016). International validation of a dithiothreitol (DTT)-based method to resolve the daratumumab interference with blood compatibility testing. *Transfusion*, *56*, 2964-2972. https://doi.org/10.1111/trf.13789

Chapuy, C. I., Nicholson, R. T., Aguad, M. D., Chapuy, B., Laubach, J. P., Richardson, P. G., et al. (2015). Resolving the daratumumab interference with blood compatibility testing. *Transfusion*, *55*(6 Pt 2), 1545-1554. https://doi.org/10.1111/trf.13069

Coluzzi, S., Londero, D., Manfroi, S., et al. (2018). Raccomandazioni per l'impiego delle metodiche in biologia molecolare in immunoematologia. Edizioni SIMTI.

De Novellis, D., Fontana, R., Giudice, V., et al. (2022). Innovative anti-CD38 and anti-BCMA targeted therapies in multiple myeloma: Mechanisms of action and resistance. *International Journal of Molecular Sciences*, *24*, 645. https://doi.org/10.3390/ijms24010645

Del Bello, A., Kamar, N., Cointault, O., et al. (2023). Anti-CD38 monoclonal antibodies interfere with isoagglutinin detection. *Transplantation*, *107*, e74-e75. https://doi.org/10.1097/TP.0000000000004453

Engstrom, C., Scherrer, E., Falconer, J., et al. (2023). A multicenter evaluation of soluble CD38 plasma pre-treatment to neutralize anti-CD38 pan-reactivity with RBCs. *34th Regional ISBT Congress*, Cape Town.

Feng, C. C., Chang, C. W., Lien, Z. Y., et al. (2023). Better resolving of anti-CD38 antibody interference with blood compatibility testing by using manual polybrene method compared with dithiothreitol-pretreatment indirect antiglobulin test. *Journal of Clinical Laboratory Analysis*, *37*, e24891. https://doi.org/10.1002/jcla.24891

Furumaki, H., Takeshita, A., Ohto, H., et al. (2021). A newly devised flow cytometric antibody binding assay helps evaluation of dithiothreitol treatment for the inactivation of CD38 on red blood cells. *Vox Sanguinis*, *116*, 725-734. https://doi.org/10.1111/vox.13052

Gessoni, G. (2024). Immunohematology. In M. Ciaccio (Ed.), *Clinical and laboratory medicine textbook* (1st ed.). Springer.

Gessoni, G., Arreghini, N., Pivetta, M., et al. (2020). Adattamento in micro colonna delle metodiche per ridurre le interferenze da daratumumab sui test pre trasfusionali. *Blood Transfusion*, *18*(Suppl 2), s72.

Gozzetti, A., Ciofini, S., Simoncelli, M., et al. (2022). Anti-CD38 monoclonal antibodies for multiple myeloma treatment. *Human Vaccines & Immunotherapeutics*, *18*, 2052658. https://doi.org/10.1080/21645515.2022.2052658

Habicht, C. P., Ridders, M., Grueger, D., et al. (2023). Mitigation of therapeutic anti-CD38 antibody interference with Fab fragments: How well does it perform? *Transfusion*, *63*, 808-816. https://doi.org/10.1111/trf.17253

Hosokawa, M., Kashiwagi, H., Nakayama, K., et al. (2018). Distinct effects of daratumumab on indirect and direct antiglobulin tests: A new method employing 0.01 mol/L dithiothreitol for negating the daratumumab interference while preserving K antigenicity (Osaka method). *Transfusion*, *58*, 3003-3013. https://doi.org/10.1111/trf.14900

Legoff, I., Dubosc-Marchenay, N., Le Bouar, M., et al. (2023). Neutralisation par la molecule sCD38 des anti-CD38 plasmatiques en immuno-hématologie; une etude multicentrique. *XXX Congrès de la Société Francophone de Transfusion Sanguine – SFTS*, Toulouse.

Lejon Crottet, S., Locher, N., Caesar, A., et al. (2023). sCD38: A soluble protein for mitigation of therapeutic anti-CD38 in the indirect antiglobulin test. *Swisstransfusion*, Rosschach.

Leleu, X., Martin, T., Weisel, K., et al. (2022). Anti-CD38 antibody therapy for patients with relapsed/refractory multiple myeloma: Differential mechanisms of action and recent clinical trial outcomes. *Annals of Hematology*, *101*, 2123-2137. https://doi.org/10.1007/s00277-022-04917-5

Matteocci, A., Coluzzi, S., De Martino, S., et al. (2023). Raccomandazioni per la gestione trasfusionale dei pazienti in trattamento con anticorpi monoclonali anti-CD38 e anti-CD47. Edizioni SIMTI.

Mei, Z., & Wool, G. D. (2019). Impact of novel monoclonal antibody therapeutics on blood bank pretransfusion testing. *Hematology/Oncology Clinics of North America*, *33*, 797-811. https://doi.org/10.1016/j.hoc.2019.05.007

National Health Service UK. (n.d.). International Blood Group reference Laboratory. Protein Development and Production Unit Bristol. Soluble recombinant CD38 (srCD38). https://ibgrl.blood.co.uk/ibgrl-research-products/recombinant-blood-group-proteins/

Nedumcheril, M. T., DeSimone, R. A., Racine-Brzostek, S. E., et al. (2021). Overcoming drug interference in transfusion testing: A spotlight on daratumumab. *Journal of Blood Medicine*, *12*, 327-336. https://doi.org/10.2147/JBM.S213510

Offidani, M., Corvatta, L., Morè, S., et al. (2021). Daratumumab for the management of newly diagnosed and relapsed/refractory multiple myeloma: Current and emerging treatments. *Frontiers in Oncology*, *10*, 624661. https://doi.org/10.3389/fonc.2020.624661

Oostendorp, M., Lammerts van Bueren, J. J., et al. (2015). When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. *Transfusion*, *55*, 1555-1562. https://doi.org/10.1111/trf.13150

Perram, J., Blayney, B., Ackerman, L., et al. (2020). Solid phase antibody screening in the presence of anti-CD38 monoclonal antibodies: A potential alternative to avoid interference. *Pathology*, *52*, 492-494. https://doi.org/10.1016/j.pathol.2020.01.683

Phou, S., Costello, C., Kopko, P. M., & Allen, E. S. (2021). Optimizing transfusion management of multiple myeloma patients receiving daratumumab-based regimens. *Transfusion*, *61*, 2054-2063. https://doi.org/10.1111/trf.16425

Pivetta, M., Vicentini, P., & Gessoni, G. (2024). Evaluation of a soluble CD38-based method for mitigation of anti-CD38 interference in pretransfusion compatibility testing. *35th Regional ISBT Congress*, Barcelona.

Shen, F., & Shen, W. (2022). Isatuximab in the treatment of multiple myeloma: A review and comparison with daratumumab. *Technology in Cancer Research & Treatment*, *21*, 15330338221106563. https://doi.org/10.1177/15330338221106563

Sullivan, H. C., Gerner-Smidt, C., Nooka, A. K., Arthur, C. M., Thompson, L., Mener, A., et al. (2017). Daratumumab (anti-CD38) induces loss of CD38 on red blood cells. *Blood*, *129*, 3033-3037. https://doi.org/10.1182/blood-2016-11-749432

Yanq, E., Mushem, I., Samarkandi, H., et al. (2023). Role of anti-CD38 monoclonal antibodies in the treatment of adult immune hematological disease. *Hematology, Oncology, and Stem Cell Therapy*, *17*, 4-12. https://doi.org/10.56875/2589-0646.1108

Zhan, Y., Wolf, L. A., Mettman, D., & Plapp, F. V. (2020). Risk of RBC alloimmunization in multiple myeloma patients treated with daratumumab. *Vox Sanguinis*, *115*, 207-212. https://doi.org/10.1111/vox.12864

Zhou, Y., Chen, L., Jiang, T., et al. (2021). 2-Mercaptoethanol (2-ME)-based IATs or Polybrene method mitigates the interference of daratumumab on blood compatibility tests. *Hematology*, *26*, 365-370. https://doi.org/10.1080/16078454.2021.1918916

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/125550