



# Real-World Evidence Data on Adverse Reactions to Infusion of Thawed Hematopoietic Progenitor Cells: Retrospective Analysis from a Single Center in India

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## Abstract

**Introduction** Adverse reactions (ARs) occur during infusion of thawed hematopoietic progenitor cells (HPCs) either due to infusion or its contents. There is sparse literature on it in the world and none in India. Therefore, we retrospectively analyzed ARs occurring during and within 1 hour of infusion of thawed HPCs.

**Objective** This study aimed to evaluate the prevalence of adverse reactions associated with the infusion of cryopreserved hematopoietic progenitor cells (HPC) and to categorize the types of adverse reactions observed in HPC transplant recipients during its infusion.

**Materials and Methods** This study was done in a tertiary-care center, between 2019 and 2022. Data collected included age, gender, diagnosis, specifications of contents of infusion product (volume of product, volume of dimethyl sulfoxide per kg body weight, total nucleated cell count per microliter, and viability of CD 34+ cells), pretreatment given, and ARs, if any from the procedure records and the hospital information system.

**Results** The present study included 55 transplant patients, and the commonest diagnosis was Hodgkin lymphoma. All were prophylactically hydrated and premedicated as per institutional protocol. AR was seen in 56.36% ( $n = 31$ ); the commonest type of ARs was nausea ( $n = 26$ ) followed by vomiting ( $n = 13$ ), abdominal pain ( $n = 4$ ), shivering ( $n = 3$ ), transient tachycardia ( $n = 2$ ), transient hypotension ( $n = 2$ ), and hematuria ( $n = 1$ ). All ARs were managed clinically by giving symptomatic treatment. No patients required intensive care, and there were no deaths or aborted procedures. Characteristics of infusion products had no significant correlation to ARs.

## Keywords

- ▶ adverse reactions
- ▶ infusion
- ▶ transplant
- ▶ hematopoietic progenitor cell

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**Conclusions** To the best of the author's knowledge, this is the first such study from India. We report an overall incidence of ARs of 56.36%, which is similar to the previously published data on ARs during thawed HPC infusions. AR is a common occurrence and can be managed medically and symptomatically.

## Introduction

Hematopoietic progenitor cell (HPC) transplant is done for various indications, both benign and malignant; benign disorders such as thalassemia major, sickle cell anemia, aplastic anemia, and malignant disorders such as acute myeloid leukemia, myelodysplastic syndromes, myeloproliferative disorders, acute lymphocytic leukemia, chronic myeloid leukemia, multiple myeloma, and lymphomas.

There are three major forms of HPC transplants performed clinically: (1) autologous transplantation, in which the patient serves as a self-donor; (2) allogeneic transplantation, from another person, and (3) cord blood transplant.<sup>1</sup> In autologous transplantation, the reinfusion of the patient's HPCs allows for the recovery of the marrow following high-dose myeloablative chemotherapy and is, hence, also known as bone marrow rescue.<sup>2</sup>

While cord HPC is infrequent, the commonest transplant in clinical settings is allogeneic HPC transplantation, where the healthy donor provides HPC either from bone marrow (HPC-M) or from peripheral blood through an apheresis procedure (HPC-A).<sup>2</sup> HPC-A has almost replaced HPC-M because of the ease and safety of collection and quicker recovery of granulocytes and platelets from the apheresis procedure compared with bone marrow collection.<sup>1</sup>

Harvested HPC-A can be stored at 4°C for up to 2 days in certain cases, such as multiple myeloma, where patient conditioning is quicker.<sup>3</sup> It must be cryopreserved in cases such as lymphoma, where patient conditioning takes 6 to 7 days or the HPC product must be shipped to a different state or country. Advancement in HPC processing over the years has led to the ability to cryopreserve cells for long-term storage, wherein stem cells can be collected in advance, cryopreserved, and then infused after the administration of myeloablative doses of chemotherapy or chemoradiotherapy in the recipients.<sup>4</sup>

Once the patient is conditioned, the HPC is infused to reconstitute the hematopoietic system. Such infusion of HPCs is generally a safe procedure, but these infusions have the potential to cause adverse reactions (ARs). These range from mild reactions such as nausea, vomiting, fever, flushing, chills, and cough to severe reactions affecting cardiovascular, respiratory, and neurological systems.<sup>5</sup> Mild ARs are more common than severe or life-threatening ARs.<sup>5</sup>

ARs due to the infusion of thawed HPCs are not well-documented globally, and there is a lack of literature on this in India. Therefore, we observed and analyzed ARs occurring

within 1 hour of infusion in transplant recipients in our retrospective cohort of 4 years.

## Materials and Methods

### Settings

This observational analytical study was conducted between 2019 and 2022 at a tertiary care hospital in India. The study population included all patients who were transfused thawed HPC during the study period.

### Collection and Cryopreservation

HPCs were collected through an apheresis (HPC-A) procedure using an automated cell separator machine (Com.Tec [Fresenius Kabi AG, Bad Homburg, Germany]) from donors (allogeneic transplant) or patients (autologous transplants). The donors/patients were mobilized using a granulocyte-colony stimulating factor with/without CXCR4 inhibitor (Plerixafor). After the target CD34 positive cell dose was achieved (4–6 million cells/kg body weight for allogeneic transplant and 2–4 million cells/kg body weight for autologous transplant), the collected HPC-A product was transported to an outside National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited Good Laboratory Practices (GLP)-certified cellular-therapy laboratory for cryopreservation. The HPC-A product was centrifuged, and excess plasma was expressed off.

The product was then transferred into freezing bags, and cryoprotectant solution (100% dimethyl sulfoxide [DMSO]) and sedimentation agent (6% hydroxyethyl starch [HES]) were added according to the product volume. The final concentration of DMSO was 5%.<sup>6,7</sup> A small aliquot (1 mL) was separated to serve as a control for the cryopreservation process. The final HPC-A product was frozen using a controlled rate freezer and then cryopreserved at less than –196°C in a vapor-phase liquid nitrogen storage freezer.

The viability of the infusion product was done twice, prefreezing and preinfusion by flow cytometry using 7-aminoactinomycin D (7-AAD). These tests were done at the same laboratory that performed the cryopreservation (NABL-accredited GLP certified). The final CD34 infusion dose was based on both postthaw viability and flow cytometry CD34 counts.

### Thawing and Infusion

On the day of the transplant, cryopreserved HPC product was transported to the transplantation center in a

temperature-monitored liquid-nitrogen cryoshipper (MVE Cryoshipper, MVE Biological Solutions, LLC, United States). The cryopreserved HPC product was thawed bedside to 37°C using a dry-plasma thawer (Barkey Plasmatherm V, Barkey GmbH & Co. KG, Germany). The process was done under sterile conditions by a transfusion medicine specialist, in the presence of a transplant physician.

The HPC infusion was performed in a positive pressure room fitted with a high-efficiency particulate air filter. All the patients were prophylactically hydrated (10–15 mL/kg body weight, up to 1 L) and premedicated with an antihistaminic (injection Pheniramine maleate 2 mL stat) and an antipyretic (Paracetamol infusion 10 mg/kg body weight, maximum dose of 1 g) as per institutional protocol, 30 minutes before the start of infusion.

The infusion was initiated through a peripherally inserted central catheter (line) in all patients immediately after thawing at the rate of 20 mL/min. The rate of infusion was increased up to 50 mL/min if the patient had no AR in the first 10 minutes. The patients were monitored for vital signs including blood pressure, pulse, respiratory rate, and oxygen saturation during and after the infusion.

#### Adverse Reaction Definition/Record

ARs were defined according to the Common Terminology Criteria for Adverse Events criteria.<sup>8</sup> Vital signs included hypotension (systolic pressure < 90 mm Hg, if previously normotensive or a decrease in systolic pressure of 20 mm Hg), hypertension (> 150/100 mm Hg if previously normotensive or an increase > 20 mm Hg in diastolic blood pressure), bradycardia (heart rate < 60 bpm), tachycardia (heart rate > 100 bpm), arrhythmia, hypoxia (oxygen saturation < 95%), tachypnoea (respiratory rate > 20), fever (temperature > 38°C), and hypothermia (temperature < 35°C).

Vital signs were recorded at the start of infusion and at 15-minute intervals thereafter till 1-hour postinfusion. Any AS occurring during and within 1-hour postinfusion was documented in the procedure sheet. Management was done according to the institutional standard operating procedures.

#### Postinfusion Protocol

Reverse barrier nursing was practiced according to the institutional protocol. Patient monitoring was done for laboratory parameters at defined frequency (complete blood counts and electrolytes once a day; liver function tests, renal function tests, and blood glucose twice a week; blood culture as and when deemed necessary). Antimicrobial prophylaxis included antibacterial (levofloxacin), antifungal (fluconazole), and antiviral (acyclovir) activities.

#### Data Collection

Data collected included patient/recipient age ( $\leq 18$ -year-old were considered in the “children” subgroup), gender, diagnosis, details of the infusion product (like volume of infusion product, volume of DMSO per kg body weight, total nucleated cell count (TNCC) per microliter, viability of

CD 34+ cells), pretreatment given, and AS, if any. The data were collected from the procedure sheet filled at the time of infusion and from the hospital information system (HIS).

#### Inclusion and Exclusion Criteria

All the patients who underwent infusion of thawed HPCs and filled procedure sheets were included in the study. Any patient with an incompletely filled procedure sheet was excluded from the study.

#### Statistical Analysis

Data were analyzed, and mean, median, and range were calculated using Microsoft Excel software and SPSS Software version 23.0 (SPSS Inc., Chicago, Illinois, United States); *p*-values < 0.05 were considered significant.

#### Ethical Approval

The study has been approved by the Institutional Ethics Committee on 29.03.2023, Reference no: 1513/2023 (Academic). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

## Results

#### Demographics and Patient Characteristics

Fifty-five patients were transfused thawed HPC-A during the study period, and all of them were included in the study analysis. There were 32 males and 23 females (M:F was 1.39:1). Twenty-nine were adult patients (52.72%), and twenty-six were children (47.27%). The most common diagnosis for which these patients were undergoing HPC transplant was Hodgkin lymphoma followed by diffuse large B cell lymphoma. All patients were transfused infusion volume on a single day.

Complete patient characteristics are mentioned in ►Table 1.

#### Characteristics of the Infusion Product

Characteristics of the thawed HPC-A product transfused to the patients undergoing transplant were studied and included the total volume of the product transfused, volume of infusion product per kg body weight, number of CD34 cells, volume of DMSO, TNCC, and viability. The median viability by 7-AAD prefreezing was 99%. The difference between median viability at prefreezing and preinfusion was 6.6% (99–92.4%). The characteristics of the infusion product are mentioned in ►Table 2.

#### Adverse Reactions

►Fig. 1 shows the incidence of AR among different study groups. The overall incidence of AR was 56.36% ( $n = 31$ ); the most common type of AS was nausea ( $n = 26$ ) followed by vomiting ( $n = 13$ ). The types of AR that occurred in the study population are demonstrated in ►Fig. 2.

**Table 1** Patient characteristics

| Patient characteristics   | n (%)         |
|---|---------------|
| Gender  |               |
| Female  | 32 (58.2%)    |
| Male  | 23 (41.8%)    |
| Age group (in years)  |               |
| ≤18   | 26 (47.27%)   |
| 19–30   | 12 (21.81%)   |
| 31–50   | 11 (20%)      |
| >50   | 06 (10.90%)   |
| Diagnosis   |               |
| Hodgkin lymphoma  | 16 (29.1%)    |
| Diffuse large B cell lymphoma   | 11 (20%)      |
| Rhabdoid tumor  | 06 (10.9%)    |
| T cell lymphoma   | 03 (5.50%)    |
| Non-Hodgkin lymphoma, Ewing's sarcoma, thalassemia major  | 02 cases each |
| Germ cell tumor, lymphoma, GI lymphoma, primary CNS lymphoma, neuroblastoma, medulloblastoma, multiple myeloma, osteosarcoma, sickle cell anemia, Wilm's tumor, ALL | 01 case each  |
| Type of transplant  |               |
| Autologous  | 49 (89.09%)   |
| Allogeneic  | 06 (10.90%)   |

Abbreviations: ALL, acute lymphocytic leukemia; CNS, central nervous system; GI, gastrointestinal.

### Management of Adverse Reactions

All AR were managed clinically, as shown in ►Table 3. No patients required intensive care, and there were no deaths or aborted procedures.

### Factors Affecting Adverse Reactions

The possible factors affecting AR in recipients like the volume of infusion product, volume of DMSO per kg body weight, TNCC per microliter, and viability of CD 34+ cells were analyzed using a chi-square test, and these had no significant correlation to AR.

**Table 2** Characteristics of the infusion product

| Characteristics                     | Mean ± SD     | Minimum | Maximum |
|-------------------------------------|---------------|---------|---------|
| Total volume transfused (mL)        | 334.8 ± 203.1 | 21.5    | 792.0   |
| Infusion product per kg body weight | 6.1 ± 3.1     | 0.6     | 19.1    |
| CD 34+ cells per kg body weight     | 7.1 ± 6.1     | 1.7     | 39.0    |
| Volume of DMSO (mL)                 | 32.4 ± 17.1   | 4.0     | 72.0    |
| TNCC × 10 <sup>3</sup> / μL         | 215.5 ± 123.4 | 9.0     | 469.0   |
| Viability (%)                       | 92.4 ± 4.6    | 76.0    | 99.0    |

Abbreviations: DMSO, dimethyl sulfoxide, SD, standard deviation; TNCC, total nucleated cell count.

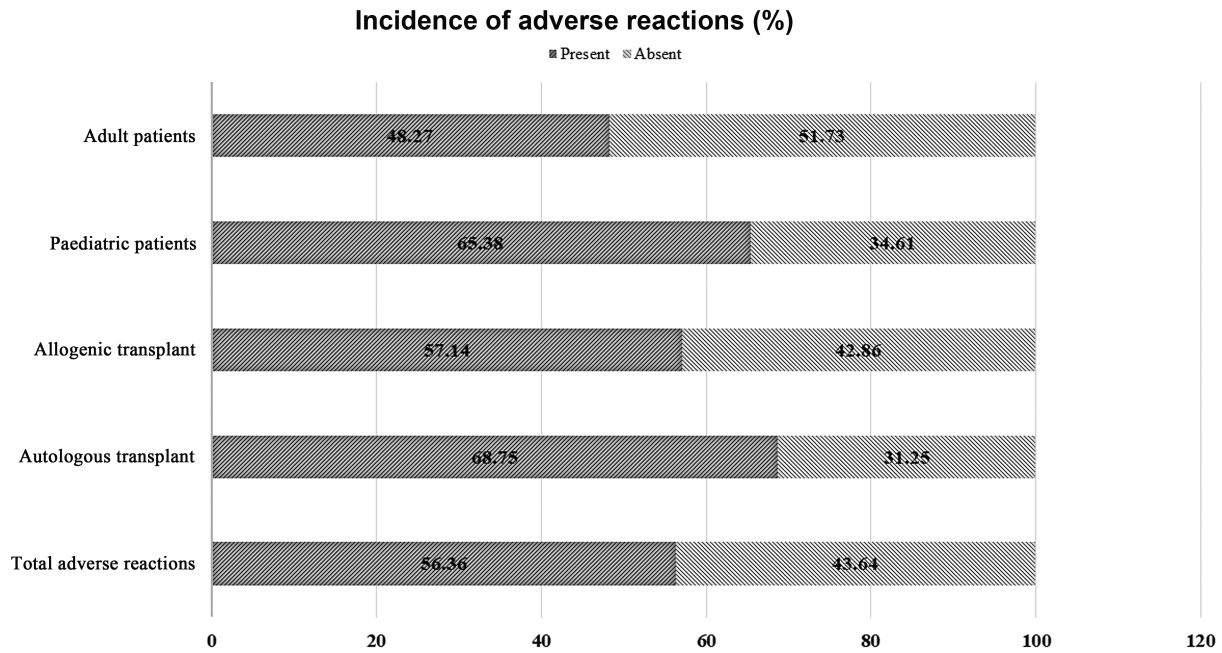
## Discussion

The present study analyzed the incidence and classified the AR occurring during the infusion of thawed hematopoietic progenitor cells. To the best of the author's knowledge, this is the first such study from India. There are several previous studies from India<sup>9–12</sup> that have reported long-term complications and transplant outcomes after infusion of cryopreserved HPC. Only one of these studies by Setia et al<sup>12</sup> briefly mentions ASs during the infusion. However, studying the ASs during the infusion of thawed HPC was not an objective of any of these studies.<sup>9–12</sup>

In the present study, the prevalence and type of ASs during and immediately after the "infusion" of thawed HPCs have been collated, analyzed, and discussed. We report an overall incidence of AR of 56.36%, which is similar to the previously published data on ASs during thawed HPC infusions.<sup>5,8,13,14</sup> In addition, 30.90% of patients had more than one AR. The most common AR reported in our study were gastrointestinal symptoms, mainly nausea (50.98%) followed by vomiting (25.49%).

Mobilized stem cells are harvested from the peripheral blood with a continuous-flow blood cell separator apheresis system (HPC-A). High-dose chemotherapy causes myeloablation of the normal marrow cells, and restoration of hematopoiesis is accomplished by infusion of HPC, thereafter. The duration from HPC-A collection to infusion might vary depending on the type of transplant and the conditioning regimen required in each case. When harvested HPC-A must be cryopreserved until the date of graft infusion, the most used cryoprotectant is DMSO,<sup>6</sup> an agent that has a known spectrum of adverse effects. ARs have been related to the amount of DMSO in the HPC-A.<sup>7</sup> Currently, there are no guidelines for the use of DMSO in stem cell cryopreservation; however, DMSO at 5% concentration is used by most centers.

Gokarn et al<sup>15</sup> studied the effect of long-term cryopreservation using 4.35% DMSO with methyl cellulose and uncontrolled rate freezing in a mechanical freezer (–80°C) on the viability of CD34+ HPCs. Twenty-six HPC harvest samples with a median cryopreservation duration of 6.6 years were studied. The median viability of post-thaw HPCs was >80% using trypan blue exclusion and flow cytometry-based 7-AAD methods. The clonogenic potential of postthaw stem cells was studied using a colony-forming unit assay, which



**Fig. 1** Incidence of adverse reactions in different study groups.

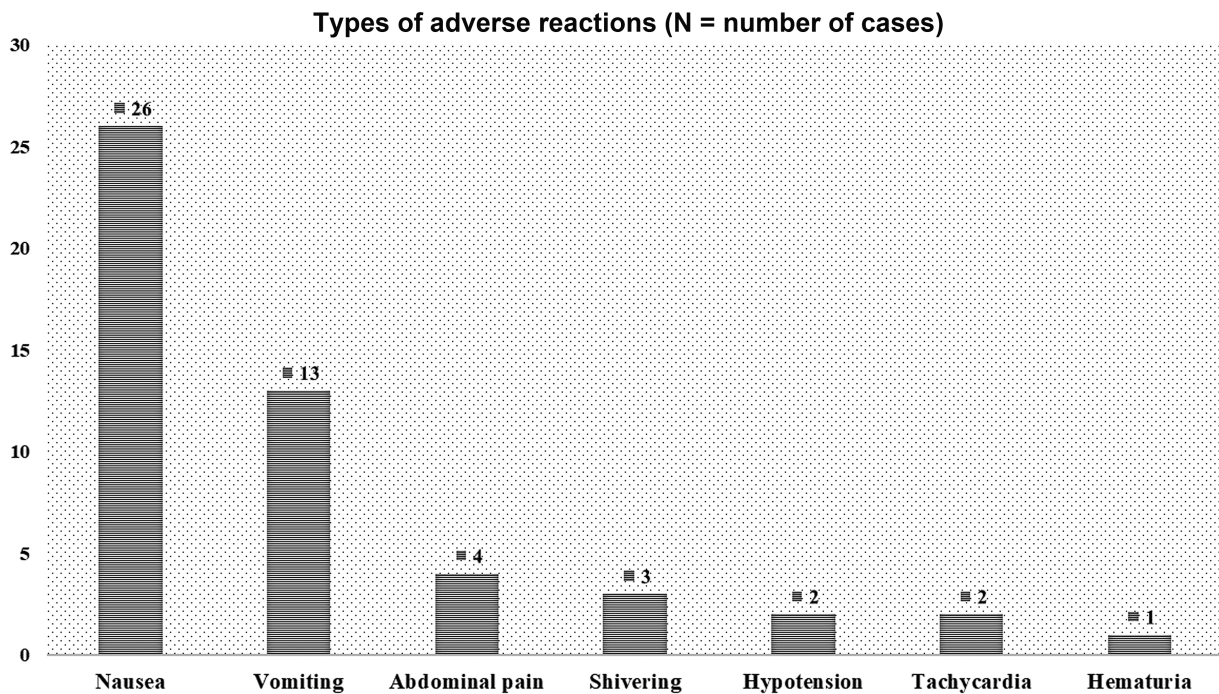
yielded a good proliferation and differentiation potential in postthaw HPCs.

#### Types of Adverse Reactions Occurring during Infusion of Thawed Hematopoietic Progenitor Cell

AR was reported in 56.36% of infusions. ▶ **Table 4** demonstrates the incidence of AR in the present study in comparison with the other published data. Among the AR reported in our patients, nausea (50.98%) was the most frequent AR followed by vomiting (25.49%). These data were in concordance with

that published by Truong et al, where they reported nausea as the most common AR (42%) followed by vomiting (28%).<sup>5</sup> Otrock et al also reported nausea and/or vomiting in 38.1% of the cases.<sup>14</sup>

Cordoba et al<sup>13</sup> reported allergic reactions as the most common AR, occurring in 43.75% cases. Similarly, Otrock et al<sup>14</sup> reported facial flushing in 39.4% of the cases. Cardiovascular symptoms were reported to be highest in the study published by Vidula et al,<sup>8</sup> in 48% of the study population. Genitourinary reactions were the least common



**Fig. 2** Types of adverse reactions in the study population.

**Table 3** Adverse reactions with corresponding management given at the time of reaction

| Adverse reaction  | Management   | Median time to resolution |
|---|--|---------------------------|
| Nausea, vomiting  | Antiemetic given stat                                      | 16 min                    |
| Transient tachycardia, transient hypotension, shivering | Slowing of infusion rate till the reaction subsided        | 28 min                    |
| Hematuria   | Double maintenance fluids given till the reaction subsides | 110 min                   |
| Abdominal pain  | Antispasmodic given stat                                   | 36 min                    |

AR in our study with hematuria occurring in 1.96% of the patients. There were no cases of reactions requiring intensive care management, no deaths occurred, and no procedure was aborted.

Some of the centers wash the cryopreserved thawed HPCs before the infusion to remove DMSO. Solves et al<sup>16</sup> studied and compared the incidence of AR in patients receiving thawed and washed HPC (peripheral blood and cord) and patients receiving noncryopreserved HPC. Before infusion, the cryopreserved HPCs were washed with a solution containing albumin, acid citrate dextrose, and dextran solution in an IBM-COBE 2991 cell processor (Gambro BCT, Lakewood, Colorado, United States). They reported a statistically insignificant difference ( $p = 0.114$ ) between the two groups and concluded that AR occurred in a significant number of patients after thawed and washed (39.2%) and noncryopre-

served (23%) HPC infusions. AR were mild, nonspecific, and well-controlled with nausea, vomiting, and fever being the most common AR.

#### Factors Affecting Adverse Reactions

Martín-Henao et al<sup>17</sup> studied the correlation of the number of granulocyte cells in the leukapheresis product to the occurrence of ARs during transfusion of thawed HPCs. They reported that the volume of DMSO kg, volume of red blood cells kg, number of nuclear cells (NCs) kg, and number of granulocytes kg in the infused graft were significant for the occurrence of AR. The grade of AR also correlated with the number of granulocytes.

Similarly, Otrrock et al found that granulocyte content was an independent risk factor for AR. Another independent predictor of AR in the same study was the volume of graft

**Table 4** Adverse reactions in the present study and comparison with published reports

| S. No. | Year of publication | Study                       | Concentration of DMSO infused | Sample size (n) | Incidence (%) | Type of adverse reactions  |
|--------|---------------------|-----------------------------|-------------------------------|-----------------|---------------|--|
| 1.     | Present study       |                             | 5%                            | 55              | 56.36%        | 50.98% ( $n = 26$ ) nausea<br>25.49% ( $n = 13$ ) vomiting<br>7.84% ( $n = 4$ ) abdominal pain<br>5.88% ( $n = 3$ ) shivering<br>3.92% ( $n = 2$ ) transient tachycardia<br>3.92% ( $n = 2$ ) transient hypotension<br>1.96% ( $n = 1$ ) hematuria |
| 2.     | 2007                | Cordoba et al <sup>13</sup> | 5–10%                         | 144             | 67.36         | 43.75% allergic reactions<br>25% gastrointestinal symptoms<br>20.83% respiratory symptoms<br>11.81% cardiovascular symptoms<br>3.47% neurological symptoms   |
| 3.     | 2015                | Vidula et al <sup>8</sup>   | 10%                           | 460             | 56.7          | 48% cardiovascular symptoms<br>14.3% respiratory symptoms<br>4.6% gastrointestinal symptoms<br>3.5% constitutional symptoms<br>1.1% neurological symptoms<br>0.22% genitourinary symptoms  |
| 4.     | 2016                | Truong et al <sup>5</sup>   | 10%                           | 213             | 55            | Most common reaction was nausea (42%), followed by vomiting (28%)  |
| 5.     | 2017                | Otrrock et al <sup>14</sup> | 10%                           | 1,269           | 37.8          | 39.4% facial flushing<br>38.1% nausea and/or vomiting<br>29% hypoxia requiring oxygen<br>16.7% chest tightness<br>12.1% cough<br>8.3% shortness of breath<br>7.3% cardiovascular symptoms  |

Abbreviations: DMSO, dimethyl sulfoxide.

infused per body weight. Infused granulocytes were significantly higher in the infusions with AR.<sup>14</sup>

For patients receiving allogeneic transplants, Vidula et al reported that the factor of greatest significance was greater red blood cell volume. They found that a greater granulocyte volume had a borderline association with the occurrence of AR.<sup>8</sup>

In the present study, the authors did not find a statistically significant correlation between characteristics of the infusion product that have previously been associated with AR, such as the number of granulocytes and DMSO volume or infusion rate and incidence of AR. A larger prospective study is needed to establish the relationship between the characteristics of the infusion product and the occurrence of AR.

## Conclusion

ASs are a common occurrence during the infusion of thawed HPCs (56.36%) and can be managed medically and symptomatically.

### Note

The manuscript has been read and approved by all the authors and the requirements for authorship have been met. Each author believes that the manuscript represents honest work.

### Author's Contributions

A.K.T. conceptualized and designed the study protocol, screened eligible studies previously published and analyzed the data. G.A. and S. Pabbi contributed to writing the report, analyzing data, and interpreting the results. S. Pawar, S. Golia, and S. Gupta contributed to extracting data from the procedure sheet and HIS, writing the report, and updating the reference lists. G.R. provided technical support during the conduct of the study. N.S. and S.Y. contributed to manuscript editing and review. All authors reviewed and approved the final manuscript.

### Patient Consent

Informed consent was obtained from each patient before commencing treatment. Patient identifiers were removed and complete confidentiality was maintained. There was no study-specific consent since anonymised data was used for this observational analysis. Institutional review board (IRB) gave a waiver for study-specific consent.

### Funding

None.

### Conflict of Interest

None declared.

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