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Retrospective Study of Implementation and Outcomes of a Non-Cryopreserved Autologous Hematopoietic Stem Cell Transplantation Program for Patients With Hematologic Malignancy in a Resource-Limited Center in Chile

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Data Collection B
Statistical Analysis C
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Manuscript Preparation E
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Background: Autologous hematopoietic progenitor cell transplantation (ASCT) is the standard treatment for many hematologic malignancies, and is usually performed with cryopreservation. However, many centers in resource-limited settings perform ASCT without cryopreservation. This retrospective study evaluated the feasibility and outcomes of implementing a non-cryopreserved ASCT program for hematologic malignancy in a public health system hospital in Chile, located far from the capital city and without local cryopreservation facilities.

Material/Methods: A retrospective study of consecutive peripheral blood ASCT procedures was performed at Hospital Base Valdivia in patients older than 15 and younger than 62 years, between June 2018 and July 2024. Data were extracted from patients' medical records. Overall survival was estimated using the Kaplan-Meier method and hazard ratios, and 95% confidence intervals were calculated using univariable Cox proportional hazards models.

Results: A total of 140 patients underwent ASCT: 81 with multiple myeloma, 31 with Hodgkin lymphoma, and 28 with non-Hodgkin lymphoma. Mobilization failure occurred in 4 patients. There was no transplant-related mortality or graft failure. Median time to neutrophil engraftment was 10.9 days, and median time to platelet engraftment was 12.3 days. Febrile neutropenia occurred in 45% of patients. Overall survival at 6 years was 83% for the entire cohort, and 96%, 88%, and 76% for non-Hodgkin lymphoma, Hodgkin lymphoma, and multiple myeloma, respectively ($P = 0.41$).

Conclusions: In this retrospective single-center public-hospital experience, non-cryopreserved ASCT was feasible without local backup cryopreservation facilities, and was associated with acceptable engraftment, early transplant-related mortality, and encouraging survival outcomes.

Keywords: Autografts • Cryopreservation • Lymphoma • Multiple Myeloma • Stem Cell Transplantation

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Introduction

Autologous hematopoietic progenitor cell transplantation, also known as autologous stem cell transplantation (ASCT) is the standard treatment for several hematologic malignancies, particularly multiple myeloma and lymphomas [1,2]. It has traditionally been performed in 4 main phases: mobilization of hematopoietic progenitor cells (HPCs) into the peripheral blood, collection via leukapheresis, cryopreservation and storage of the graft, administration of high-dose conditioning chemotherapy, and graft infusion, followed by inpatient supportive care until hematologic recovery. The cryopreservation is performed to maintain the viability of collected HPCs until the patient is ready to undergo conditioning and transplantation [3]. This approach, however, requires substantial investment in infrastructure, equipment, and technical expertise for cryopreservation and long-term storage, which poses significant challenges for centers with limited resources or those located far from major metropolitan areas [4].

In many Latin American public health systems, there are few ASCT centers, limiting patients' access to this therapy despite an increasing demand [5]. Non-cryopreserved ASCT has emerged as an alternative strategy that is already in use in several centers worldwide. This approach can reduce the cost and complexity of the procedure and, in many settings, has helped to narrow access gaps [6]. A comparative study from a national private center shows that performing transplants without cryopreservation is safe and equally effective [7].

The key question is whether a non-cryopreserved ASCT program can be implemented in public regional centers distant from major capitals and cryopreservation facilities, while maintaining acceptable outcomes in terms of mobilization, complications, and survival. Here, we present a study that describes the implementation of an ASCT program at a regional public hospital lacking a backup cryopreservation center. In this study, we evaluated mobilization, engraftment, complications, and overall survival in patients with multiple myeloma and lymphoma, using a retrospective, single-center design based on a review of medical records and survival analysis via Kaplan-Meier and Cox models.

Material and Methods

Study Design and Population

We performed a retrospective study at Hospital Base Valdivia that included patients older than 15 and up to 61 years of age who underwent ASCT between June 2018 and July 2024. For this analysis, we included all patients assigned to our center by the National Transplant Program, which initiated the HPC mobilization. Patients who declined transplantation or did not start mobilization because of relapse or other reasons were excluded.

The study was approved by the Research Ethics Committee of the Valdivia Health Service, exempt resolution No. 018120 dated September 15, 2023, and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Data Collection

Demographic, clinical, biochemical, microbiological, flow cytometry, and treatment data were collected from medical records. Outcomes and event dates (engraftment, infections, intensive care admission, relapse, and death) were also obtained from chart review.

Mobilization and Collection

All ASCT procedures utilized peripheral blood-derived HPCs. Mobilization was performed using filgrastim (10-16 µg/kg/day, tailored to the risk of mobilization failure) for 5 days. On day 4, if the peripheral blood CD34+ count was < 15 cells/µL, patients received plerixafor (0.24 mg/kg). Either 1 or 2 leukapheresis sessions were conducted to achieve a target total CD34+ cell yield of $\geq 2 \times 10^6$ cells/kg of recipient body weight.

Apheresis was performed with the Spectra Optia™ Apheresis System (Terumo BCT). Acid-citrate-dextrose formula A (ACD-A) was used for extracorporeal circuit anticoagulation, with a whole-blood-to-ACD-A ratio of 14: 1, in accordance with the manufacturer's recommendations. To prevent hypocalcemia, 250 mL of 0.9% normal saline plus 4 grams of calcium gluconate were administered via intravenous infusion over 4 hours. CD34+ cell counts were measured using a BD FACSCanto II flow cytometer and the BD Stem Cell Enumeration Kit (in-vitro diagnostic protocol). The collected HPC product was stored at 3 to 5 °C in a dedicated refrigerator for red blood cell bags in the hospital blood bank.

Conditioning Regimens

After adequate HPC collection, conditioning was initiated. For multiple myeloma, conditioning consisted of melphalan 200 mg/m^2 administered over 30 minutes, or 140 mg/m^2 for patients with creatinine clearance < 30 mL/min. For lymphoma, patients received carmustine-etoposide-cyclophosphamide conditioning: carmustine 300 mg/m^2 on day -4, etoposide 150 mg/m^2 every 12 hours on days -4, -3, and -2, and cyclophosphamide 1500 mg/m^2 on days -4, -3, -2, and -1, with day 0 defined as the day of HPC infusion,

Inpatient Care and Graft Infusion

All patients were hospitalized on the day of HPC collection in a HEPA-filtered positive-pressure isolation unit. A double-lumen central venous catheter (CVC) was inserted and removed immediately after HPC infusion. For multiple myeloma patients,

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Table 1. Descriptive analysis: age, sex, and diagnosis of patients undergoing autologous hematopoietic progenitor cell transplantation at Valdivia Base Hospital, June 2018-July 2024.

	All patients	Multiple myeloma	Hodgkin lymphoma	Non-Hodgkin lymphoma
Number of patients	140	81	31	28
Average age, years (range)	47.2 (16-61)	52.1 (34-61)	38.8 (16-60)	43.1 (23-60)
Male sex, n (%)	71 (50.7)	40 (50.6)	15 (48.3)	15 (55.5)

the graft was infused the day after collection. For lymphoma patients, infusion was performed on day 5, at least 36 hours after completion of the last etoposide dose.

During hospitalization, patients received prophylaxis with oral acyclovir 400 mg every 12 hours and oral levofloxacin 500 mg once daily until the absolute neutrophil count (ANC) exceeded 1.0×10^3 cells/μL, and oral fluconazole 150 mg once daily until 1 week after discharge with ANC > 1.0×10^3 cells/μL. After the 73rd transplant, dexamethasone 4 mg/day orally was added as prophylaxis for engraftment syndrome from day 7 until discharge. Filgrastim 300 μg subcutaneously was administered from day 5 until ANC > 1.0×10^3 cells/μL was achieved. In cases of febrile neutropenia, empiric antibiotic therapy consisted of meropenem 1 g intravenously every 8 hours plus vancomycin 2 g loading dose followed by 1 g intravenously every 12 hours. Transfusions were given according to local protocols, using irradiated blood products when available.

Patients were discharged once they achieved ANC > 0.5×10^3 cells/μL and platelet count > 20×10^3 cells/μL without transfusion requirements. They also had to have adequate oral intake, and any active infection or complication had to be under control.

Outcomes

The outcomes studied were hematopoietic engraftment, transplant-related mortality, overall survival, mobilization and collection metrics (CD34+ cell dose, use of plerixafor, mobilization success or failure), and the duration of hospitalization following transplantation. Adverse effects evaluated included the rates and characteristics of febrile neutropenia, intensive care unit admission, engraftment syndrome, the need for parenteral nutrition, and the occurrence of mucositis greater than grade III.

Definitions and Statistical Analysis

Neutrophil engraftment was defined as the number of days from HPC infusion (day 0) to the first of 3 consecutive days with ANC > 0.5×10^3 cells/μL. Platelet engraftment was defined as the number of days from infusion to the first of 7 consecutive days with platelet count > 20×10^3 cells/μL without platelet transfusions in the preceding 48 hours. Transplant-related mortality was defined as any death within 100 days after the ASCT procedure.

Categorical variables were expressed as frequencies and percentages. Continuous variables were reported as averages and ranges. Overall survival was estimated using the Kaplan-Meier method, and survival curves were stratified by underlying disease using RStudio (RStudio PBC, Boston, MA, USA). Hazard ratio (HR) and 95% confidence interval (CI) were calculated using univariable Cox proportional hazards models for the selected covariates. Multivariable Cox analysis was not performed because no additional covariates showed a statistically significant association with overall survival in univariable analyses.

Results

Patient Characteristics

Between June 2018 and July 2024, 140 patients underwent ASCT at Hospital Base Valdivia. Underlying diagnoses were multiple myeloma in 81 patients, Hodgkin lymphoma in 31 patients, and non-Hodgkin lymphoma in 28 patients. Baseline patient and disease characteristics are summarized in **Table 1**. The outcomes of the 4 patients who experienced mobilization failure are reported separately and were not included in the main transplant outcome analysis.

Mobilization and Cell Yield

The mean CD34 + cell dose collected was 4×10^6 cells/kg, with a minimum of 1.37×10^6 cells/kg and a maximum of 13.3×10^6 cells/kg. Plerixafor was required in 76 patients (54.2%). Among lymphoma patients, 74% required plerixafor, compared with 41.7% of multiple myeloma patients. Mobilization failure occurred in 4 patients (2 with multiple myeloma and 2 with lymphoma). Two of these patients successfully underwent remobilization with cryopreservation at another center using a single day of collection, whereas the remaining 2 were confirmed as mobilization failures and did not proceed to transplant.

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Table 2. Descriptive analysis: time to engraftment, length of hospital stay, episodes of febrile neutropenia, and infectious foci according to underlying diagnosis in patients undergoing ASCT.

	Multiple myeloma	Hodgkin lymphoma	NonHodgkin lymphoma
Neutrophil engraftment, average days (range)	10.6 (10-13)	11.2 (10-13)	11.5 (10-14)
Platelet engraftment, average days (range)	11.5 (9-14)	12.0 (8-23)	16.2 (10-100)
Discharge, average days postinfusion (range)	12.2 (10-24)	13.4 (10-23)	15.7 (10-45)
Febrile neutropenia, n (%)	27 (34.0)	20 (64.5)	15 (55.0)
Day of onset, average days (range)	6.6 (4-9)	6.1 (1-11)	6.3 (1-10)
No identified focus, n (%)	6 (22.2)	10 (50.0)	7 (46.6)
Abdominal focus, n (%)	10 (37.0)	5 (25.0)	4 (26.6)
Cutaneous focus, n (%)	5 (18.5)	4 (20.0)	1 (6.6)
Perianal focus, n (%)	1 (3.7)	2 (10.0)	1 (6.6)
Mixed focus, n (%)	1 (3.7)	1 (5.0)	1 (6.6)
CVCrelated focus, n (%)	3 (11.1)	2 (10.0)	0
Respiratory focus, n (%)	0	0	1 (6.6)
Clostridioides difficile diarrhea, n (%)	3 (11.1)	0	0

ASCT, autologous stem cell transplantation; CVC, central venous catheter.

Hematologic Recovery and Infectious Complications

No patient experienced graft failure or transplant-related mortality, defined as death within 100 days after ASCT. The average time to neutrophil engraftment was 10.9 days (range 10-14), and average time to platelet engraftment was 12.3 days (range 8-100). The average length of hospital stay post-transplant was 13.1 days (range 10-45). Engraftment times and rates of febrile neutropenia by underlying disease are shown in **Table 2**. Febrile neutropenia occurred in 63 patients (45%), with an average onset of 6.6 days after transplant (range 0-11). No infectious focus was identified in 23 patients (36.5%). Among those with an identified focus, the most frequent sites were abdominal (19 patients, 30.1%), cutaneous (10 patients, 15.8%), catheter-related (5 patients, 7.9%), perianal (5 patients, 7.9%), and *Clostridioides difficile*-associated diarrhea (3 patients, 4.7%); and 3 patients (4.7%) had mixed foci. Two patients had positive blood cultures. No patient required parenteral nutrition, and no mucositis greater than grade II was observed. Six patients developed engraftment syndrome requiring corticosteroid therapy. Two patients required admission to the intensive care unit: 1 for engraftment syndrome requiring mechanical ventilation, and 1 for septic shock due to febrile neutropenia. They both required vasopressors for 1 day and then returned to the transplant unit.

Survival

At 6 years, overall survival for the entire cohort was 83%. By disease subgroup, overall survival was 96% for non-Hodgkin lymphoma, 88% for Hodgkin lymphoma, and 76% for multiple myeloma ($P = 0.41$) (**Figures 1, 2**).

Univariable Cox regression showed a strong trend toward higher mortality in patients who required plerixafor for mobilization (HR 2.91; 95% CI 0.87-9.75), although this did not reach statistical significance ($P = 0.07$) (**Figure 3**). There was no significant difference in mortality between patients with and without febrile neutropenia (HR 1.12; 95% CI 0.37-3.37) ($P = 0.84$) (**Figure 4**).

Discussion

In this retrospective study of ASCT without cryopreservation, we observed that neutrophil and platelet engraftment occurred at a median of 11 and 12 days, respectively; no graft failures were recorded, and 6-year overall survival was 83% (96% for non-Hodgkin lymphoma, 88% for Hodgkin lymphoma, and 76% for multiple myeloma).

In many Latin American public health systems, the number of ASCT centers is insufficient to meet the growing demand for

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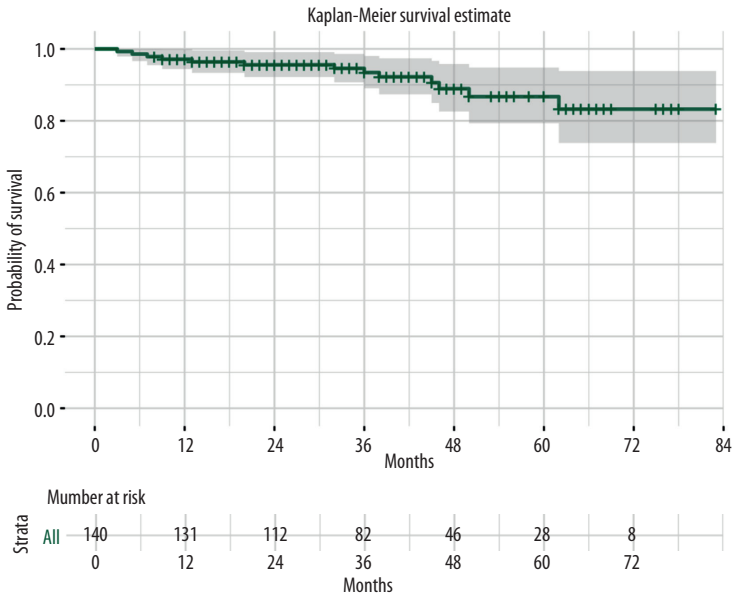

Figure 1. Overall survival of all patients undergoing autologous hematopoietic progenitor cell transplantation.

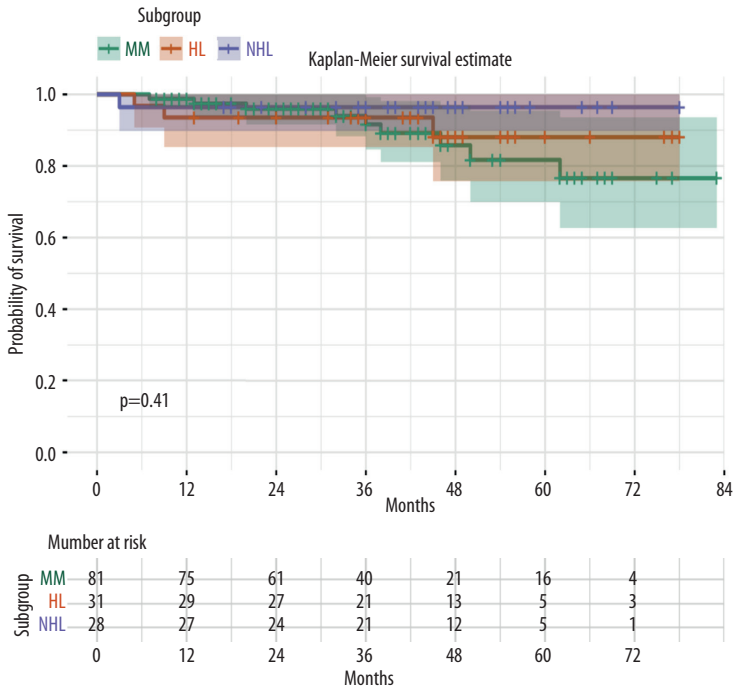

Figure 2. Overall survival according to underlying disease in patients undergoing autologous hematopoietic progenitor cell transplantation. MM, multiple myeloma; HL, Hodgkin lymphoma; NHL, nonHodgkin lymphoma.

transplantation [5]. Establishing a transplant program outside the capital typically requires cryopreservation facilities, and therefore entails high implementation and maintenance costs. Non-cryopreserved ASCT is a valid alternative that has been adopted in some low- and middle-income countries and in selected private centers to reduce costs. Several reports have shown comparable or even shorter durations of pancytopenia and similar or fewer complications compared with conventional cryopreserved approaches [7-13].

Our experience, although retrospective, suggests that implementing a non-cryopreserved ASCT program in a public hospital

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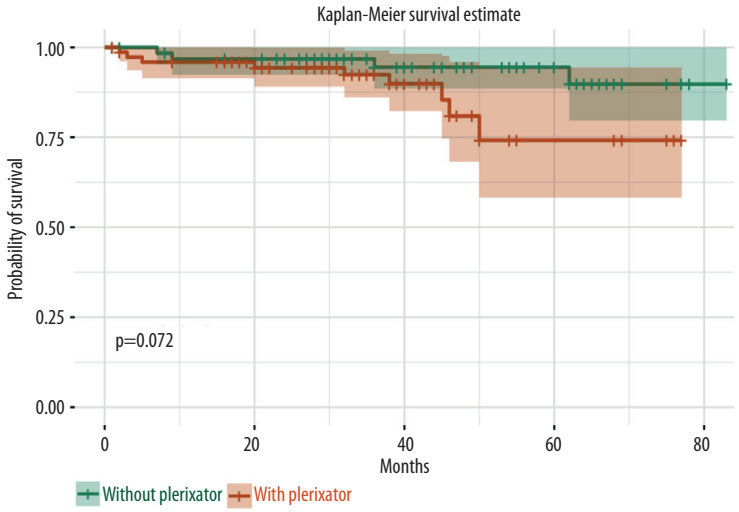

Figure 3. Mortality according to plerixafor requirement.

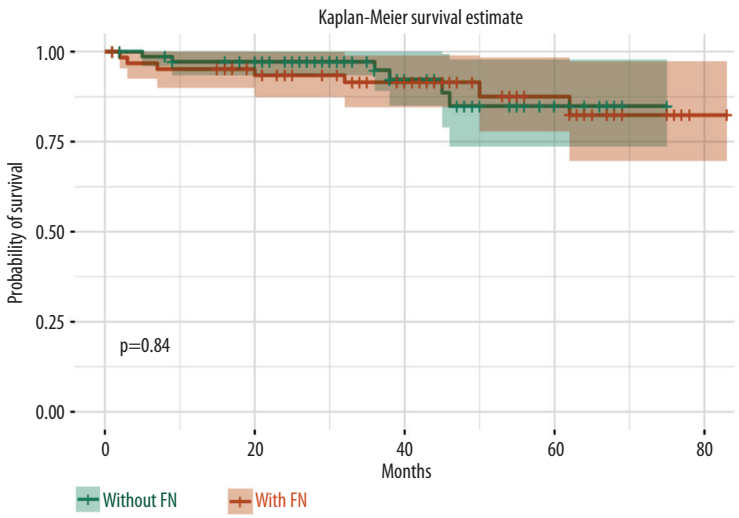

Figure 4. Mortality according to febrile neutropenia. FN, febrile neutropenia.

located far from the capital and from the nearest cryopreservation center (approximately 850 km away), with the goal of decentralizing care and improving patient access, is feasible. This requires strict coordination across all participating teams and adherence to predefined, non-modifiable timelines for mobilization, collection, conditioning, and infusion. Despite these logistical constraints, our outcomes are similar to those reported in large transplant centers, with median neutrophil engraftment between 10 and 12 days, no graft failure, and very low transplant-related mortality in both myeloma and lymphoma [7-13]. Overall survival by disease group was also comparable to published data for standard ASCT [14]. When comparing results by disease type with data published by other centers in Latin America or within Chile, we observe that for multiple myeloma patients, overall survival is similar to that reported by private centers (80% at 5 years) and even better than that reported by public centers (70%), though the latter figure may be influenced by access to new treatments [15]. Regarding lymphomas, the results are similar to those published for both Chilean healthcare systems [16,17].

The absence of transplant-related mortality in our cohort may be partly explained by the national inclusion criteria of the Chilean ASCT program, which restricts eligibility to patients with multiple myeloma achieving at least very good partial response or complete remission and an upper age limit of 60 years. In contrast, many published series include older patients, often up to 65 years or more.

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When comparing outcomes by disease, we observed that ASCT for multiple myeloma was associated with higher CD34+ cell yields and fewer complications than ASCT for lymphoma. Infections were almost 50% less frequent in myeloma patients (34% vs 59.7%), and neutrophil and platelet engraftment occurred sooner. These findings suggest that initiating a non-cryopreserved ASCT program in regional centers distant from cryopreservation facilities with multiple myeloma patients, and subsequently expanding to lymphoma, may be a reasonable stepwise strategy for new programs.

When evaluating all experiences, including our own, the question arises as to whether ASCT in myeloma patients requires cryopreservation. Even in centers with this technology available, this issue raises a hypothesis that should be addressed in comparative studies [18].

Our outcomes are also comparable to those reported for fully outpatient ASCT programs, indicating that an outpatient or early-discharge model could be implemented in the future to further reduce costs and increase transplant capacity in hospitals lacking dedicated inpatient transplant units [19]

Mobilization failure occurred in 4 patients, 2 of whom were in relapse at the time of mobilization. The other 2 patients eventually achieved successful mobilization at a cryopreservation center. Although mobilization failure is less of a limitation today, given the broader availability of plerixafor, it should still be considered for very high-risk patients, who should be referred to centers with cryopreservation capability whenever possible.

This study has several limitations. It is a retrospective, single-center analysis subject to potential selection bias and incomplete data collection. The sample size is relatively modest for subgroup analysis. As no contemporary control group with cryopreserved samples was included, it is not possible to infer the comparative efficacy of the non-cryopreservation strategy.

These results reflect the experience of a regional public hospital integrated into a national transplant program; therefore, they may not be fully generalizable to other settings.

Conclusions

In this retrospective single-center public-hospital experience, non-cryopreserved ASCT was feasible and was associated with acceptable engraftment, low early transplant-related mortality, and encouraging survival outcomes. These findings support this approach as a practical option for selected regional centers seeking to expand transplant access, while broader comparative and implementation claims should be confirmed in additional settings.

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Patient Permission Declaration

Patient permission was obtained.

Declaration of Figures' Authenticity

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