Clinical and Experimental Obstetrics & Gynecology

Original Research

Diminished ovarian reserve and ectopic ovaries in patients with Mayer-Rokitansky-Küster-Hauser syndrome candidates for Uterus Transplantation: our experience

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DOI:10.31083/j.ceog4804143

Submitted: 31 March 2021 Revised: 16 April 2021 Accepted: 17 May 2021 Published: 15 August 2021

Background: Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a congenital disease characterized by variable degrees of utero-vaginal agenesis. A diminished ovarian reserve and ectopic ovaries are common in these patients. The objective of this study is to highlight the issues relating to assisted reproduction in patients with MRKH syndrome who are candidates for Uterus Transplantation and to describe alternative methods to the classic transvaginal oocyte retrieval. *Methods*: In Italy, the Research Project for Uterus Transplantation from brain-dead donor started on 14/06/2018 (n. 1438/CNT2018). The potential recipients underwent to clinical evaluation, laboratory and instrumental tests to achieve eligibility for transplantation, ovarian stimulation and oocytes retrieval at the Obstetrics and Gynecology Unit of the Cannizzaro Hospital of Catania (Italy). A retrospective analysis of all patients affected by MRKH syndrome who are candidates for uterus transplantation was performed in order to highlight the problems encountered in assisted reproduction due to poor ovarian reserve or extra-pelvic gonads. *Results*: 15 of 64 patients (23%) were excluded after the first visit due to the poor ovarian reserve and/or ectopic ovaries. A 27-years old patient with MRKH syndrome type 2, with extra-pelvic ovaries and good ovarian reserve underwent ultrasound-guided and laparoscopically assisted transvaginal and transabdominal oocyte pick-up, with total retrieval of 12 oocytes and no short or long-term complications. *Conclusion*: Laparoscopically assisted oocyte retrieval is a feasible technique that can overcome some limitations for inclusion on waiting list for Uterus Transplantation, such as ectopic ovaries.

Keywords

Assisted reproduction; Laparoscopy; MRKH syndrome; Oocyte pick-up; Uterus transplantation

1. Introduction

Mayer-Rokitansky-Küster-Hauser (MRKH) Syndrome is a congenital disorder of the female genital tract, described for the first time in 1829 by Mayer. The estimated prevalence is approximately 1 in 4500 live births, and it is the second most common cause of primary amenorrhea [1]. The etiology is still unknown, including genetic and environmental factors. Most cases are sporadic, although some authors suggest autosomal dominant inheritance, with incomplete penetrance and variable expressivity. The syndrome is characterized by variable degrees of utero-vaginal agenesis [2] (type 1), in some cases with urinary, skeletal, auditory and cardiovascular systems disorder (type 2). Patients generally have a normal female karyotype (46 XX), normal secondary sexual characteristics and normal ovarian function [3]. However, a frequent finding in patients with the Syndrome is a diminished ovarian reserve (reduced blood levels of Anti-Mullerian Hormone, AMH) [4] and dislocated ovaries [5]. AMH is expressed by the granulosa cells of the small developing follicles, from the primordial follicles to the stage of pre-antral follicles and small antral follicles (up to 8 mm in diameter). Although AMH levels show high inter-individual variability in women, intra-individual variability is mainly a function of age, with minimal fluctuations during the menstrual cycle.

Uterus transplantation represents the surgical treatment of absolute uterine factor infertility and it is the only option to achieve a pregnancy in women with congenital or acquired absence of the uterus: transplantation of the uterus is not intended as a "quoad vitam", but it is a "quoad valetudinem" transplant, that is only aimed at obtaining a pregnancy.

The aim of this study is to highlight the issues related to ovarian stimulation and oocyte retrieval in patients with MRKH syndrome who are candidates for Uterus Transplantation and to describe alternative methods to classic transvaginal aspiration, often not possible in these patients.

2. Materials and methods

On 14 June 2018, in Italy, the National Transplant Center approved the Research Project for Uterus Transplantation from brain-dead donor (n. 1438/CNT2018), authorising a team of researcher from the Gynecology and Obstetrics Unit of the "Cannizzaro" Hospital of Catania and the Organ Transplant Center of the "Policlinico-Vittorio Emanuele" Hospital of Catania. The Project was approved by Ethical Committee Catania 1 of the "Policlinico-Vittorio Emanuele" Hospital of Catania (protocol n. 0026684 on 3 July 2017).

Since the date of approval of the study, we have received 151 requests for inclusion on the waiting list. In accordance with the American Society of Reproductive Medicine [6], inclusion criteria are: patients aged between 18 and 40, suffering from AUFI for congenital causes (e.g., MRKH syndrome) or acquired (e.g., hysterectomy for obstetric complications or for benign pathology). Patients hysterectomized for malignant neoplasms, patients with hepatitis B/C or Human Immunodeficiency Virus (HIV) positive, patients with Human Papilloma Virus (HPV) DNA Test positive, patients with low ovarian reserve (AMH <1 ng/mL) and patients with acquired absence of uterus who have already had at least one child were excluded.

The donor is a brain-dead subject between the age of 18 and until menopause. The uterus is explanted, regardless of the donor's obstetric history, in the absence of obvious macroscopic pathologies (e.g., uterine fibromatosis); the previous cesarean section is an exclusion criterion.

The donor must also meet the donor eligibility criteria set out in the "Protocol for the suitability of the donor of solid organs" [7] of the National Transplant Center. The Montreal Criteria [8] must also be met in the recipient, the donor and the medical team.

The patients who, following a preliminary evaluation of the clinical history and the documentation provided, were found to comply with the inclusion criteria, were summoned to the Unit of Obstetrics and Gynecology of the Cannizzaro Hospital in Catania, to perform the first visit, blood tests (liver and renal function, coagulation, infectious disease tests), hormonal tests (follitropin, luteotropin, estradiol, progesterone and AMH), blood group, gynecological examination with pap smear, vaginal swab and HPV DNA test, transvaginal ultrasound, psychological counseling and interviews with the signing of informed consent.

The inclusion on the waiting list is the last of several steps to obtain gynecological and surgical eligibility. The second step is ovarian stimulation in order to retrieve and cryopreserve oocytes before transplantation to ensure ovarian function prior to invasive prolonged surgery and because immunosuppressive therapy after surgery potentially decreases the gonadal response [9]. Internationally recognized target is a minimum number of 10 good quality blastocysts but since embryo cryopreservation is banned in Italy, a retrieval of at least 10 oocytes has been identified as a requirement for inclusion in the waiting list, in one or more stimulations.

Eligibility for assisted reproduction was evaluated with AMH and transvaginal ultrasound to detect ovarian location in order to define the accessibility to vaginal retrieval.

Partners of potential recipients underwent spermogram and semen culture. Due to a lack of menstrual cycles in patients with MRKH syndrome, a long type stimulation protocol was performed. The hypothetical secretory phase of the menstrual cycle was identified by performing hormonal assays (estradiol, progesterone, follitropin, luteotropin). Patients were down-regulated with Gonadotropin Releasing Hormone (GnRH) agonist Buserelin 1 mg/die (Suprefact; Sanofi-aventis) and received 150 IU Urinary-human Follicular Stimlating Hormone (u-hFSH) daily (Fostimon; Institut Biochimique SA (IBSA)) for the first four days and then dose was modified (until to 225 IU) according to ovarian response monitored by ultrasound and hormonal assessment. Ovulation was induced by a single administration of human chorionic gonadotropin (hCG) (7.500 IU; Gonasi®, IBSA, Lugano, Switzerland) on the day after the last u-hFSH injection when the leading follicle had reached a mean diameter of $\geq$16 mm. Aspiration of the oocytes was performed transvaginally by ultrasound guidance 36 hours after hCG administration. Oocytes were denuded 2 hours after oocyte retrieval. Following the nuclear maturity evaluation, only the metaphase II (MII) oocytes were selected for immediate vitrification. All the vitrification solutions were obtained from Fertipro (Beernem, Belgium) and oocytes vitrification was carried out performing the Fertivit cooling and using the Cryolock (CRYOLOCK®, Alpharetta, GA, USA) as device. Briefly, after 2 minutes into the pre-incubation medium, oocyte were exposed to a mixture of ethylene glycol and dimethylsulfoxide (1.25%, 2.5%, 5%, 10% v/v), and, as final step, oocytes were subjected to a vitrification solution (20% v/v) of the same cryo-protectants for 40–60 seconds and then vitrification was induced by immediate plunging into liquid nitrogen. Two to three oocytes (maximum) were loaded per Cryolock. Oocytes were stored in liquid nitrogen tanks.

We retrospectively analyzed all patients affected by MRKH syndrome who are candidates for uterus transplantation in order to highlight the problems encountered in ovarian stimulation and oocyte pick-up due to diminished ovarian reserve or extra-pelvic ovaries. A patient underwent laparoscopic assisted oocyte pick-up due to ectopic ovaries not accessible with vaginal approach; this patient is the recipient of the first uterus transplant in Italy performed in Catania on 21 August 2020.

Table 1 summarizes inclusion criteria by steps to transplant.

3. Results

Since the approval of research project we received 151 applications for uterus transplantation (Fig. 1), summarizes screening algorithm with exclusion criteria for each step. 39 patients were immediately excluded after application for age $>$40 years (26) or previous cancer (13). After the first visit 15 of 64 patients were excluded for AMH <1 ng/mL and ectopic ovaries not accessible with transvaginal approach; 5 of them had both low AMH and ectopic ovaries (Table 2). Among these patients, the median age was 29 years, the median value of AMH was 0.5 ng/mL.

A patient with extra-pelvic ovaries but good ovarian reserve underwent laparoscopically assisted transvaginal oocytes pick-up. The patient, V.A., aged 29, was affected from MRKH syndrome with uterine agenesis and vagi-

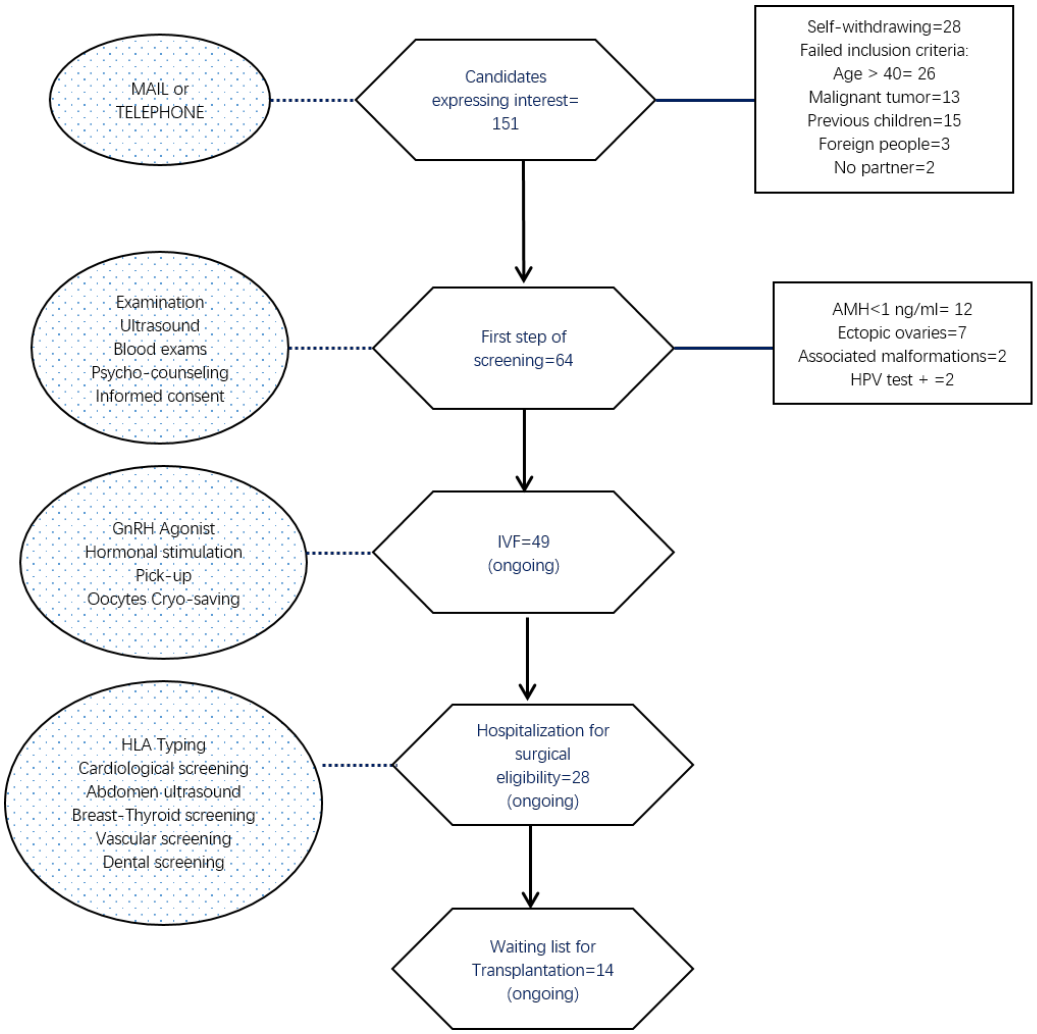

Fig. 1. Results of screening for candidates.

nal hypoplasia corrected by dilation therapy, with associated left renal agenesis and normal renal function. Personal history was negative for medical disease and surgery, karyotype was 46 XX. Vaginal canal was 6 cm long, ovaries were not detectable by transvaginal ultrasound but only by transabdominal probe: the structure and ovarian size were normal, as were the antral follicle count (AMH = 10.8 ng/mL). The blood group was A Rh positive. In consideration of the personal history and good health, given the good ovarian reserve although with extra-pelvic ovaries, the patient was declared eligible to continue with the next steps. The patient was down-regulated with GnRH agonist Buserelin 1 mg/die (Suprefact; Sanofi-aventis.) and received 150 IU u-hFSH daily (Fostimon; IBSA) for the first four days and 225U u-hFSH daily (Fostimon; IBSA) until the day before hCG somministration. The patient received gonadotropin treatment for a 11 days and ovulation was induced by a single administration of human chorionic gonadotropin (hCG) (7.500 IU; Gonasi®, IBSA, Lugano, Switzerland) on the day after the last u-hFSH injection when the leading follicle had reached a mean diameter of $\geq$16 mm. Aspiration of the oocytes was performed transvaginally by ultrasound guidance 36 hours after hCG administration. In consideration of extra-pelvic ovaries, oocytes pick-up was performed by laparoscopic assistance (**Supplementary Video**). The patient was placed in the lithotomy position under general anesthesia with oro-tracheal intubation. Pneumoperitoneum was created by "open" technique and two ancillary 5 mm trocars were inserted in the medium abdomen bilaterally. Uterus was rudimentary with two small horns closely adjacent to each ovary (Figs. 2,3). Ovaries, despite increased in volume following hormonal stimulation, were located above the bifurcation of the iliac vessels. Small bowel and colon were pushed up in order to reduce risk of iatrogenic perforation. Ovaries were push down by atraumatic forceps in order to reduce the distance to vagina, through which oocytes retrieval was performed by 17 G 1.4 mm needle placed on transvaginal ultrasound probe aspiration kit (Origio). Some follicles were retrieved also by the right ancillary trocar under direct vision and ultrasound control (Fig. 4). 21 oocytes were aspired, 12 of them were deemed eligible to immediate vitrification and cryopreservation. Surgical time was 40 minutes and no early

Table 1. Inclusion criteria according to steps of screening.

Steps of screening	Inclusion criteria
First contact	· Age 18–40 years · No children · Stable partnership · No hysterectomy for malignant tumors
First examination	· Normal renal/hepatic function · Normal hemostasis · No HIV, HCV, HBV infections · No severe comorbidities · No HPV genital infection · No azoospermia in the partner · No ectopic ovaries · AMH >1 ng/mL
IVF	· At least 10 frozen oocytes
Surgical eligibility	· Normal echocardiogram · Normal chest x-ray · Normal abdmominal ultrasound · Norma vascular screening · Normal dental function · No breast or thyroid lesions

Notes: HIV, Human Immunodeficiency Virus; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; HPV, Human Papilloma Virus; AMH, Anti Mullerian Hormone; IVF, *In Vitro* Fertilization.

and late complications were reported. The patient was discharged on post-surgical day 1.

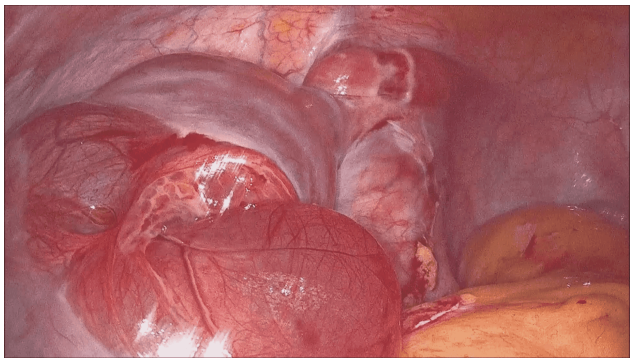

Fig. 2. Left ovary. Left ovary increased in volume with rudimentary left uterus.

4. Discussion

Uterus transplantation, although still considered an experimental technique, is now a real option for the treatment of AUFI [10–15], and it is a rapidly expanding field in many countries [16]. Almost 90% of uterus transplant recipients are affected by MRKH syndrome [9].

The aim of this study is to highlight the issues regarding assisted reproduction in patients with MRKH syndrome who are candidates for Uterus Transplantation. In these patients it is common to find ectopic ovaries, diminished ovarian reserve and low response to hormonal stimulation; vascular factors determine ectopic place and diminished ovarian reserve. The issues regarding *in vitro* fertilization would be more serious in the patients affected from type II MRKH syndrome (ovarian or renal malformations) than type I (isolated utero-vaginal hypoplasia) [17].

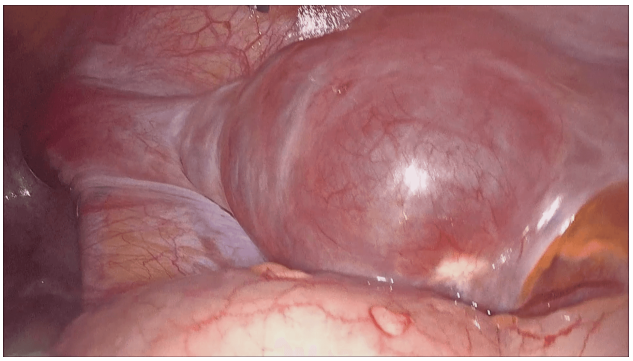

Fig. 3. Right ovary. Right ovary increased in volume with rudimentary right uterus.

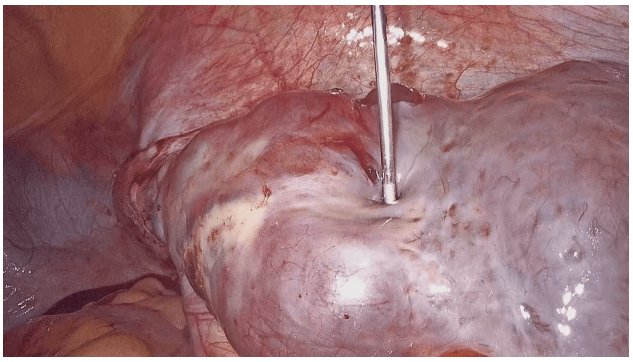

Fig. 4. Transabdominal pick-up. Transabdominal oocytes aspiration under direct vision control.

Ectopic ovaries in MRKH syndrome are reported in literature from 15 to 42 % [18]. This discrepancy could be due to different definition and criteria for the term "ectopic ovaries" in the various series.

About preliminary steps in the patients candidates to uterus transplantation, Akan [19] reports that 22 of 119 MRKH syndrome patients had ovaries placed in subcostal position. Brannstrom [13] describes issues about starting and monitoring ovarian stimulation due to lack of menstruation and more cranial and lateral ovarian site compared to healthy controls. Czech experience [20], instead, reports only 3 patients with extra-pelvic ovaries, in which oocytes retrieval was performed by standard transvaginal technique. Also the French Project doesn't report low ovarian reserve and dislocated ovaries between not eligible patients [21].

Imaging has a key role during preliminary steps to transplantation and in general for MRKH syndrome patients. Transvaginal and transabdominal ultrasound are useful to study ovarian reserve by counting antral follicles, to detect patients affected from atypical syndrome with ectopic ovaries

Table 2. Data of not eligible MRKH patients.

Patient	Age (years)	BMI (Kg/m^2)	AMH (ng/mL)	Vagina	Ovaries	Other malformations and comorbidities
1	23	22	0.5	Vecchietti	Ectopic	Right kidney agenesis
2	31	25	0.3	Native	Pelvic	Premature ovarian failure (FSH = 74 mUI/mL)
3	25	21	0.1	Native	Pelvic	Right salpingo-ovariectomy
4	26	21	0.5	Native	Ectopic	Renal deficiency
5	35	26	0.5	Native	Ectopic	HPV 16
6	26	21	0.2	Vecchietti	Ectopic	Pelvich Horseshoe Kidney
7	21	20	3.5	Vecchietti	Ectopic	HPV 31
8	29	23	0.4	Vecchietti	Pelvic	
9	38	29	0.1	Native	Pelvic	Left kidney agenesis
10	39	25	0.5	Native	Ectopic	
11	27	23	1.0	Vecchietti	Ectopic	
12	37	26	1.1	Vecchietti	Ectopic	
13	29	20	0.4	Vecchietti	Pelvic	Left kidney agenesis
14	35	28	0.9	Native	Pelvic	Renal deficiency
15	39	25	0.4	Vecchietti	Pelvic	

and urinary malformations. Magnetic resonance is important for diagnosis, ovarian localization and preparation to uterus transplantation [22].

Oocytes retrieval depends on ovarian position. In the course of assisted reproduction history, oocytes retrieval has remarkably changed [23]. The laparotomy sampling, initially performed, was followed by laparoscopic sampling and, almost simultaneously, by ultrasound-guided sampling, first trans-abdominal and then trans-vaginal. Laparoscopic sampling, for some years the almost universally used method, had the advantage of ensuring optimal vision of the operating field and performing a complete diagnostic exploration of the pelvis during oocyte retrieval; this technique had some disadvantages such as the need for general anesthesia with orotracheal intubation, the greater invasiveness compared to the ultrasound-guided procedure and, finally, the potential toxicity for the oocyte exposed to carbon dioxide.

Relating to *in vitro* fertilization prior to uterus transplant, it is known that other methods of oocytes retrieval alternative to the transvaginal one have been taken into consideration. Brannstrom [13] reports abdominal ultrasound-guided pick-up in a patient affected from atypical MRKH syndrome with unilateral kidney agenesis.

Our retrospective analysis and in particular the case report, demonstrates that laparoscopy can play a role in the diagnosis but also in the therapy of patients with MRKH syndrome (laparoscopic neo-vagina), and also in oocyte retrieval with the classic technique [24]. The diagnostic role of laparoscopy is already known: most of the Italian patients affected from MRKH syndrome candidates to Uterus Transplantation underwent laparoscopy; many of them, especially those with neovagina, will undergo diagnostic laparoscopy prior to transplant in order to study surgical anatomy. Laparoscopically assisted transvaginal and/or transabdominal pick-up [25] is a feasible technique allowing to overcome anatomical limits (short vagina, extra-pelvic ovaries) and making the patient eligible. This study is limited by the number of cases, as the research project has been active for a few months and the MRKH syndrome is a rare disease. Another limit is that only one uterus transplant has been performed in Italy, therefore obstetric outcomes are not yet available in patients suffering from MRKH syndrome undergoing assisted reproduction techniques at our hospital.

5. Conclusions

We believe, according to the experience gained in the recruitment of these patients, that the factors listed above, such as poor follicular reserve and ovarian position, represent an important limitation to be considered as a further obstacle to achieving the main objective of the research: obtaining a pregnancy.

Patients affected from MRKH syndrome, in addition to variable degrees of uterus and vaginal aplasia, often have altered ovarian location and function in terms of ovarian reserve and response to hormonal stimulation.

Imaging and laparoscopy play an important role either in diagnosis and therapy. Laparoscopically assisted transvaginal and/or transabdominal ultrasound-guided oocytes pick-up is a feasible technique and represents a valid alternative to classic transvaginal pick-up in the patients have short vagina and extra-pelvic ovaries.

Author contributions

BP and MV wrote the manuscript. PS, GS and PB performed surgery. MV, PV and PB performed the research on laboratory. GS, PV and PS performed ideation and supervision of manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Project was approved by Ethical Committee Catania 1 of the "Policlinico-Vittorio Emanuele" Hospital of Catania (protocol n. 0026684 on 03/07/2017). All the patients signed informed consent.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://ceog.imrpress.com/EN/10.31083/j.ceog4804143.

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