



Laparoscopic varicocelectomy: Does intraoperative lymphography with vital dye influence the outcome?

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Summary

Introduction

Vital dyes lymphography during laparoscopic varicocelectomy (LV) has been suggested to allow for lymphatic mapping thereby allowing for lymphatic sparing (LS) and subsequent reduction of hydrocele formation. Intratesticular injection of the dye seems to achieve the best lymphatic visualization, however, the potential toxicity of some agents, is a cause of concern for the risk of testicular pathological changes. We report our experience with LV, to test the hypothesis that Intraoperative lymphography with methylene blue (BL), is safe and reduces the rate of post-operative hydrocelectomy. Secondary aim was to evaluate the effect of intratesticular injection of the dye.

Material and methods

Retrospective review of all consecutive patients undergoing LV < 18 years of age, between 2018 and 2022, and with at least 12-month follow-up. We compared hydrocele rate after LS-LV vs. non-LS-LV and between patients undergoing LS with vs. without BL and according to injection site. Post-operative ultrasounds were reviewed to rule out testicular anomalies in patients undergoing BL. Reoperations for varicocele were also noted.

Results

116 patients undergoing LV at a median (range) age of 14 (10–17) years were included. Post-operative hydrocelectomy rate was significantly more common ($p = 0.033$) in patients undergoing non-LS (3/38,

8 %) vs. LS (0/78) LV. No hydrocelectomy was performed in patients undergoing LS-LV with (0/47) or without (0/31) BL. Vital dye injection site was intratesticular in 21/47 (45 %) cases. Avascular intratesticular lesions were identified in 4 (19 %) patients and persisted after a median (range) follow-up time of 21 (14–28) months. Two varicoceles were reoperated for recurrence, one in each group.

Discussion

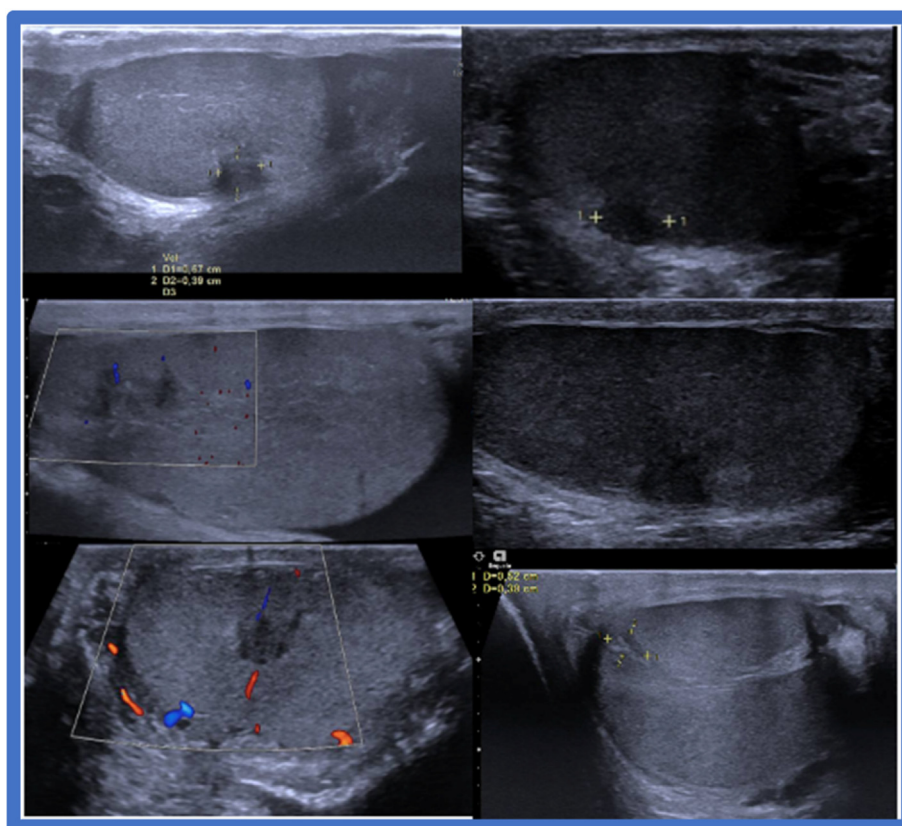
Vital dyes lymphography improves lymphatics visualization during LV. Although, in present study, overall hydrocele rate was not significantly different in patients undergoing LS vs. non-LS procedures, hydrocelectomy was required only after non-LS procedures. In keeping with other 2 recent studies, avascular intratesticular lesions were observed in one fifth of cases undergoing Intratesticular dye injection. These lesions did not change during follow-up, but their clinical significance remains to clarify. Limitations of study include the retrospective design and the small number of patients undergoing intratesticular dye injection, but still this is one of the largest series with long-term follow-up of intratesticular lesions after BL.

Conclusions

LS-LV significantly reduced the risk of hydrocelectomy, irrespective to the use of BL. Intratesticular methylene blue injection was associated with persistent testicular lesions on follow-up ultrasounds in 19 % of cases. More studies are necessary to prove the safety of dye injections into the testis.

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Summary figure Testicular abnormalities after intra-parenchymal injection of Methylene Blue dye.

Introduction

Post-operative hydrocele formation is the most common complication after adolescent varicocelectomy. Reportedly, prevalence ranges from 0 to 30 % and the surgical technique seems to possibly play an important role [1–6]. For laparoscopic varicocelectomy (LV), risk of hydrocele formation seems to be higher after en-bloc ligation of the spermatic vessels. Therefore, lymphatic-sparing (LS) techniques have been described [5–7]. Intraoperative lymphography can help sparing the lymphatics and different techniques for intraoperative lymphatic mapping have been described. In 2001, Oswald et al. first described the intra-scrotal injection of isosulfan blue to enhance intraoperative identification of the lymphatics to be spared [6]. Variations of the technique include the use of different vital dyes such as methylene blue or, more recently, indocyanine green, and, different injection sites such as intratesticular and intra-scrotal ones [8,9]. Intratesticular injection seems to be the most effective way to perform the lymphography allowing for 100 % of successful mapping vs. the 80 % achieved with intra-scrotal injection [8–10]. However, intratesticular dye injection also carries the concern of testicular changes, although series focusing on this issue are scant [7,9]. An animal study documented varying degrees of testicular histological changes after injection of substances into the testis including saline solution [4]. Particularly for methylene blue, local or systemic reactions including skin and fat necrosis have been

described in the large experience for sentinel lymph nodes detection in breast cancer [11]. However, the clinical effect of the injection of methylene blue into the testis is still to be studied.

Over the last years, we have used an intraoperative lymphography with methylene blue injected either in the scrotum or in the testis. Aim of present study was to report our experience with LV with and without lymphatic mapping and sparing, and to verify its safety, by assessing possible side effects of either intra-scrotal or intratesticular injection of the dye. Our testing hypothesis was that LS-LV with methylene blue is safe and increases the chance to avoid post-operative hydrocele formation and need for hydrocelectomy.

Material and methods

We retrospectively analyzed electronic dossier of all consecutive pediatric patients undergoing laparoscopic varicocelectomy at our institution from January 2018 to December 2022.

Patients older than 18 years old, with follow-up shorter than 12 months, or with incomplete data were excluded from the study.

Preoperative evaluation included clinical examination and scrotal doppler ultrasound. Varicocele severity was graded clinically according to Dubbin and Amelar classification [12]. Ultrasound was used to assess testicular volume

and the doppler for hemodynamic assessment of the retrograde blood flow.

Indications for varicocelectomy included testicular asymmetry with the affected testicle at least 20 % smaller than the contralateral one, associated symptoms including orchialgia or scrotal discomfort, and bilaterality.

All surgical procedures were performed by eight pediatric urologist and were laparoscopic non-artery sparing Palomo procedures. The procedure is performed with three 5-mm trocars, a trans-umbilical optic, and 2 operating instruments one in the pelvis and the other in the right iliac fossa. A 1–2 cm wide window is created into the peritoneum about 2 cm above the internal inguinal ring to access the spermatic vessels. Vessels are closed with endo-clips or hemlocks, based on surgeon preference.

The technique used for the management of the lymphatics varied based on surgeon preference, including:

- Non-Lymphatic sparing Laparoscopic varicocelectomy (Non-LS-LV), i.e. en-bloc ligation of all the vessels without any dissection
- Lymphatic sparing Laparoscopic Palomo varicocelectomy without dye lymphography (No-DL-LS-LV). In these cases, the sparing of lymphatics was achieved by skeletonization of the vessels and preservation of all the fatty tissue around the vessels without any attempt to identification of the lymphatics;
- Lymphatic sparing Laparoscopic Palomo Varicocelectomy with dye lymphography (DL-LS-LV).

Lymphography was performed using a solution of 1 ml of methylene blue diluted with 4 ml of saline solution. The injection was performed after anesthetic induction and before trocars placement. The solution was injected with a 23-G needle either 2 ml intra-scrotal or 1 ml intratesticular. Intratesticular injection was obtained introducing the needle for 1 cm into the testicular body. Intra-scrotal injection was performed on the lateral side of the scrotum in the space between the dartos and the tunica vaginalis. We used Methylene blue for lymphography as it is easily available, inexpensive and least anaphylactogenic [11]. We have chosen the dilution to 20 % of the product to reduce inflammatory reactions [4].

Patients undergoing Non-LS-LV were compared to those undergoing LS-LV. In addition, patients receiving intra-scrotal injection were compared to those undergoing intratesticular dye injection. Patients were followed up by physical examination at, 1, 6 and 12 months after the procedure. Ultrasound was performed between 6 and 12 months in all patients with intratesticular injection of methylene blue or in case of suspected hydrocele or varicocele recurrence. In case of testicular anomalies, the US was performed after 1 month and every 6 months. Semen analysis was not evaluated for the young age of most of our patients.

End points for study included varicocele recurrence, post-operative hydrocele formation, post-operative hydrocelectomy, and systemic reactions or local scrotal/testicular lesions after dye injection.

The hydrocele was classified into grade I, II and III following postoperative hydrocele definition used by Zundel et al. [12]. Grade I and II were considered sub-clinical

hydroceles and followed up conservatively, surgery was instead recommended in grade III hydrocele persisting more than 6 months after LP. Post-operative testicular ultrasounds were reviewed in patients undergoing intratesticular injection to rule out possible intratesticular lesions.

Intratesticular lesion were defined as hypoechogenic/hyperechogenic intraparenchymal alteration not previously observed in the preoperative ultrasonogram.

Data were analyzed using the PASW Statistics Program v. 20.0 (IBM Corp., Armonk, NY, USA) for MacOSX. Continuous variables were reported as median (IQR) and categorical variables as frequency (percentage). Non parametric tests were used for comparison between groups. Differences were considered significant for a $p < 0.05$.

Results

During the study period, 116 patients underwent LV at a median (range) age of 14.6 [13–16] years old. All patients had clinically grade III varicocele. Median (range) post-operative follow up was 18 (12–58) months.

A Non-LS-LV was performed in 38/116 (33 %) patients, whereas the remaining 78/116 (67 %) patients underwent a LS-LV. Of the latter, 31/78 (40 %), underwent only skeletonization of the cord without BL, whereas 47/78 (60 %) underwent methylene BL. The methylene blue was injected into the scrotum in 26/47 (55 %) and in the testicle in 21/47 (45 %) (Fig. 1).

Overall, the varicocele recurred/persisted post-operatively in 7/116 (6 %) patients, without difference between patients undergoing Non-LS-LV vs LS-LV, $p = 0.07$. Two patients (1.7 %) required reintervention, eventually, one in each group.

Hydrocele developed in 17/116 (15 %) patients overall. Median (range) follow-up after LV in these patients was 28 (12–45) months. Hydrocele was grade I in 11/17 (64.7 %), grade II in 3/17 (17.6 %) and grade III in 3/17 (17.6 %) patients (Table 1). The overall hydrocele rate was not statistically different ($p = 0.2$) in patients undergoing Non-LS-LV vs. LS-LV, 4/38 (11 %) vs 13/78 (17 %), respectively. Likewise, hydrocele rate was not significantly different ($p = 0.2$) in patients undergoing varicocelectomy with vs. without BL, 6/47 (13 %) vs. 7/31 (23 %), respectively, or in patients undergoing intra-scrotal vs. intratesticular injection 3/26 (12 %) vs. 3/21 (14 %), $p = 0.2$ (Table 2).

Grade III hydrocele occurred in 3 patients who underwent hydrocelectomy a median (range) of 21 (14–28) months from LV. Grade III hydrocele was significantly more common ($p = 0.033$) in patients undergoing Non-LS-LV vs. LS-LV, 3/38 (8 %) vs. 0/78 (0 %), respectively, whereas it was not significantly different between patients undergoing LS-LV with vs. without blue lymphography, (0/47) vs. (0/31), respectively.

No post-operative systemic or local complications were observed in patient undergoing BL. Of the 21 patients undergoing intratesticular injection, 4 (19 %) had evidence of an intra-parenchymal avascular hypoechogenic or calcified area on the follow-up scrotal ultrasound (Fig. 2). These patients were followed up with serial testicular ultrasounds, and the lesion remained unchanged after a median (range) follow-up time of 21 (12–27) months from the

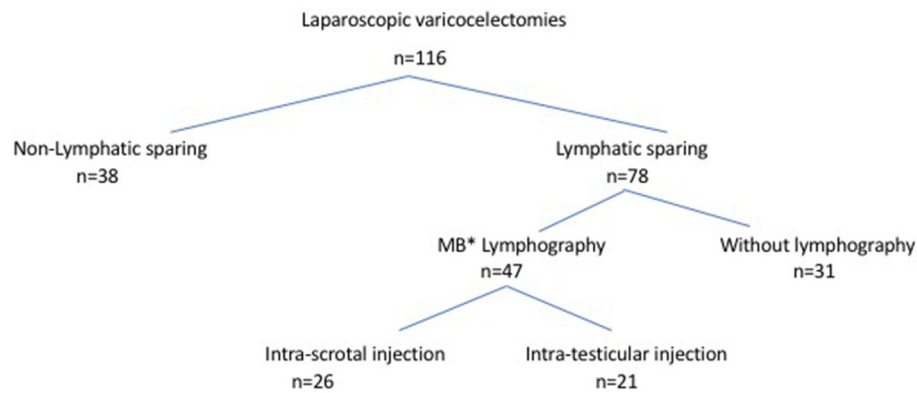


Fig. 1 Surgical techniques for laparoscopic varicocelectomy. *Methylene Blue.

Table 1 Hydrocele classification.

Patients (N°)	age at surgery (y.o)	Surgical Technique	Hydrocele US (classification and diameter in mm)	Hydrocele Examination	Hydrocele Grading system second Zundel	Hydrocelectomy (timing in months)	Follow-up time	Persistence
1	12	No BL-LS-LV	Small ^a	Small scrotal asymmetry	II	No	45	Yes
2	12	No LS-LV	Small ^a	Not palpable	I	No	44	
3	17	BL-LS-LV	Small ^a	No palpable	I	No	42	
4	15	No BL-LS-LV	Small; 11 mm	Not palpable	I	No	42	Yes
5	12	BL-LS-LV	Small ^a	Not palpable	I	No	28	
6	14	BL-LS-LV	Small; 18 mm	Small soft hydrocele	II	No	17	
7	14	No BL-LS-LV	Small; 5 mm	Not palpable	I	No	29	
8	15	BL-LS-LV	Small ^a	Not palpable	I	No	37	
9	14	No BL-LS-LV	Small ^a	Not palpable	I	No	24	
10	15	No BL-LS-LV	Small; 10 mm	Not palpable	I	No	23	
11	17	No BL-LS-LV	Small; 6 mm	Not palpable	I	No	12	
12	15	No BL-LS-LV	Small; 8 mm	Not palpable	I	No	19	
13	15	BL-LS-LV	Small; 15 mm	Small soft hydrocele	II	No	12	Yes
14	16	BL-LS-LV	Small ^a	Not palpable	I	No	12	
15	14	No LS-LV	Large ^a	Large firm hydrocele	III	28	42	
16	12	No LS-LV	Large ^a	Large firm hydrocele	III	22	26	
17	16	No LS-LV	Large:30 mm	Huge firm hydrocele	III	14	27	

Abbreviations: US: Ultrasonogram; LV: laparoscopic varicocelectomy; LS: Lymphatic sparing; BL: blue lymphography.

^a No US diameter.

Table 2 Hydrocele comparison between groups.

Procedures	Patients (n°; %)	Hydrocele (n°; %)	P	Hydrocelectomy (n°; %)	P
Non LS-LV	38/116 (33 %)	4/38 (11 %)	0.2	3/38 (8 %)	0.033
vs	vs	vs		vs	
LS-LV	78/116 (67 %)	13/78 (17 %)		0/78 (0 %)	
LS-LV without BL	31/78 (40 %)	7/31 (23 %)	0.2	0/31 (0 %)	/
vs	vs	vs		vs	
LS-LV with BL	47/78 (60 %)	6/47 (13 %)		0/78 (0 %)	
Intra-testicular	21/47 (45 %)	3/21 (14 %)	0.2	0/21 (0 %)	/
vs	vs	vs		vs	
Intra-scrotal	26/47 (55 %)	3/26 (12 %)		0/26 (0 %)	

Abbreviations: LV: Laparoscopic varicocelectomy; LS: Lymphatic sparing; BL: Blue lymphography.

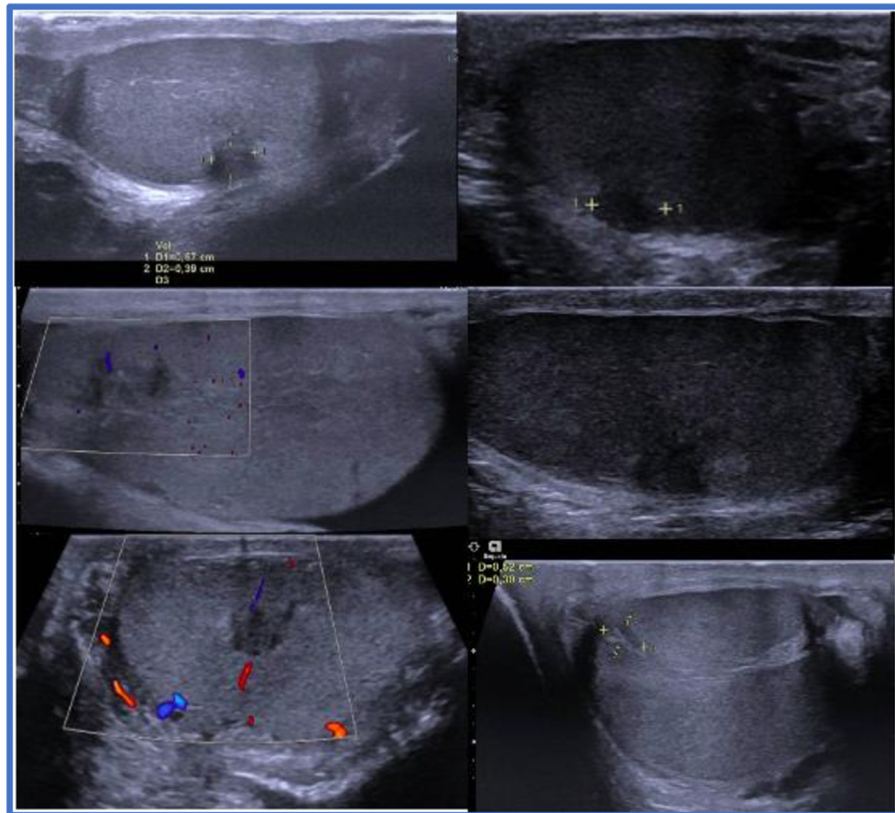


Fig. 2 Testicular abnormalities after intra-parenchymal injection of Methylene Blue dye.

initial diagnosis (Table 3). All these patients are still on follow-up.

Discussion

Laparoscopic Palomo varicocelectomy was effective with <2 % rate of varicocele requiring redo surgery. Hydrocele formation was the most common complication. Hydrocele rate was not significantly different in patients undergoing LS vs. non-LS procedures. Severe hydroceles requiring additional surgery, however, occurred only after non-LS procedures. Intratesticular injection of the vital dye for lymphatic mapping was associated with avascular intratesticular changes in one fifth of cases.

Laparoscopic varicocelectomy is the most common technique used for adolescent varicocele. Hydrocele formation is the major complication. Different modifications have been proposed to improve results, but still no standard technique exists [1–3].

Looking to the literature, lymphography, regardless of the dye, increases the possibility of visualization and sparing of lymphatic vessels, but, the injection site (dartos, vaginal or intraparenchymal), could influence the success of lymphatic visualization and the incidence of post-operative hydrocele [1].

Intratesticular injection seems to be the most effective method. Consistently, intraoperative visualization has been reported in 80 % of cases after intra-dartos injection of the dye and 100 % after intratesticular injection. Of note,

Table 3 Ultrasonogram intraparenchymal anomalies.

Patient (N°)	Age (y.o) ^a	Lesion site	Lesion diameter (mm)	Lesion US description	Follow-up	Persistence
(1)	17	Medial testicular	1,0	No vascularized intraparenchymal hypoechoic, inhomogeneous.	27	Yes
(2)	15	Medial testicular	1,5	No vascularized intraparenchymal hypoechoic, inhomogeneous with calcification	12	Yes
(3)	14	Medial testicular	8	No vascularized intraparenchymal calcification	19	Yes
(4)	15	Ill medial	8	No vascularized intraparenchymal hypoechoic, inhomogeneous	25	Yes

^a Year old.

however, post-operative hydrocele was reportedly 0 % of in both cases [8].

Despite the highest rate of successful mapping, intratesticular injection is a cause of concern for the risk of testicular changes. The effect of methylene blue on tissues is controversial, its anti-oxidant, anti-apoptotic and neuroprotective effect has been reported in an experimental porcine model of cardiac arrest and cerebral ischemia [16]. In its large use for sentinel lymph node biopsy, cases of anaphylaxis, fat necrosis, and other allergic reactions have been reported, even if seems to be safer comparing with other dyes with less systemic reactions [11–17].

In keeping with the literature on varicocelelectomy, we did not observe any systemic reaction or allergies, the only reaction we observed after methylene blue injection was a transitory skin coloration [15]. Lack of such adverse events in our experience can possibly be explained by the fact that our patients underwent a single injection of a limited and diluted amount of dye.

In our series 1 every 5 patients undergoing intratesticular injection presented during follow-up with asymptomatic nonpalpable lesions. These lesions were always avascular at doppler evaluation, sometimes were calcified, and persisted after a median (range) follow-up of 21 (12–27) months from diagnosis. In a series of 72 patients receiving intratesticular injection of 2 ml of indocyanine blue, Esposito et al. observed similar lesions in 3 cases (4.2 %). In contrast to our cases, two of the 3 lesions resolved within 12 months [9]. To prevent the complication, the authors recommended reducing the dosage of injected indocyanine to 1 ml reporting no influence on lymphatics detection. Hung et al. instead, found testicular changes in six patients undergoing sub-dartoid injection of 2 ml of methylene blue. The authors attributed these changes to an inadvertent injection into the testicular parenchyma [7].

Of note, some of our lesions include calcifications. We hypothesized that this could be explained by inflammation or tissue necrosis at the injection site. The absence of vascularization and the stability or sometimes reduction in diameter, explain the benign nature of these anomalies. Using animal models Makari et al. studied the effect of intratesticular injection of different vital dyes including isosulfan blue, methylene blue and trypan blue. The authors found some degrees of histopathological changes in all testicles injected with vital dyes including in those injected with normal saline. Intratesticular changes ranged from dystrophic calcifications, to tubular atrophy, necrosis and hyalinization. Authors concluded that the increasing intratesticular pressure could explain the changes which was unrelated to the concentration, volume and the type of vital dye [4].

More important the clinical consequence of these lesions remains unclear. Long-term follow-up study including data on semen analysis in patients with such lesions are necessary to understand the effect of these substances on testicular function.

Post-operative hydrocele can occur after varicocelelectomy because of a disruption of lymphatic outflow from the testis [4,6]. Therefore, a lymphatic-sparing surgery should in principle avoid such complication. Consistently, in our patients, severe hydroceles, namely grade III hydrocele requiring

hydrocelelectomy, occurred only in patients undergoing non-lymphatic sparing LV. The overall prevalence of hydrocele, instead, was not differ between patients undergoing Non-LS-LV vs. LS-LV. In our opinion, a transitory alteration of lymphatic outflow following the manipulation of the cord during surgery can account for these transitory hydroceles.

If one aims to a lymphatic-sparing varicocelelectomy, it might seem intuitive a clear identification of the lymphatic vessels to be key. Consistently, different vital dyes have been tested to achieve an intraoperative mapping of the lymphatics. Tested substances include methylene blue, isosulfan blue and patent blue V [4,6]. More recently, indocyanine green (ICG) using the fluorescence technology with Firefly system has been introduced. In the preliminary data results are good in term of low varicocele incidence and no post-operative hydrocele in intratesticular injection [9].

Drlik et al. suggested the intraoperative lymphography to be unnecessary, as the magnification of the laparoscopic camera would allow for direct visualization of the lymphatic vessels [14]. In our experience and opinion, while this can be true under certain circumstances, it is difficult to duplicate for every surgeon and every patient, and the lymphatic mapping is anyway time-saving. Rather, our data, in keeping with Glassberg et al. support the principle that a lymphatic-sparing procedure could be achieved just skeletonizing the spermatic vessels and preserving the peripheral fat where the lymphatic vessels are embedded, irrespective of intraoperative mapping of the lymphatic vessels [5].

If a lymphatic mapping is anyway elected, several variables should be considered, including type of dye, injected dose, timing of injection and injection site. Esposito et al. recommended injecting <0.5 ml of isosulfan blue, as larger amounts can cause a blue coloration of the entire bundle making lymphatic dissection difficult. Still, no post-operative hydrocele was reported in their series [9].

This study has limitations. Due to the retrospective design, some data were missing, especially ultrasound data. Procedure where done following our clinical practice and not standardized. The choice between the type of procedure and the injection site were at surgeon discretion. Considering the young age of our patients semen analysis was not possible to obtain. Finally, only few patients underwent intratesticular injection.

On the other hand, to our knowledge, this is a large series and the one reporting the longest follow-up in testicular anomalies after intratesticular injection of blue dyes for varicocelelectomy.

Conclusion

Laparoscopic lymphatic sparing Palomo varicocelelectomy is an effective method to reduce post-operative complications in children regardless to the surgical technique. However, our result suggests no clear benefit of lymphography for lymphatic sparing procedures. Lymphography with methylene blue was safe without systemic or local reactions. Nevertheless, intratesticular injection of vital dyes should be used carefully because long-term effects on testicular function still unknown. Longer follow-up is

necessary to prove the safety of its intratesticular injection.

Authors' contributions

Ana Ludy Lopes Mendes have given substantial contributions to the conception, the design, data acquisition, analysis, interpretation, and the drafting of the manuscript. Giulia D'Ippolito and Andrea Celeste Barneschi given contribution to data acquisition and revision, Marco Castagnetti, contributed to the design, interpretation of the data, the drafting and revised the manuscript critically. Ermelinda Mele, Michele Innocenzi, Giuseppe Collura and Laura del Prete participate to data acquisition and analysis. All authors read and approved the final version of the manuscript.

Ethical statement

Ethical statement was not request for this study.

Declaration of Generative AI and AI-assisted technologies in the writing process

No AI has used to draw-up this manuscript.

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