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ORIGINAL PAPER

Is rapid preparation for thyroidectomy in severe Graves' disease beneficial? The relationship between clinical and immunohistochemical aspects

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Abstract *Background* To evaluate the effect of Lugol's Iodine (LI) on angiogenic characteristics and postthyroidectomy morbidity in patients with Graves' Disease (GD). *Methods* Medical records of 153 patients, who underwent thyroidectomies for GD between 1987 and 2006 were reviewed. LI in addition to antithyroid drugs (ATD) was administered to 16 toxic Graves' cases (group I), while 137 received ATD only (group II). In order to evaluate thyroid vascularity, immunohistochemical staining (IHS) for VEGF and CD31 for assessing microvessel density (MVD) were performed in group I, 20 random cases of group II, and 7 stochastic patients with non-toxic goiter (group III). *Results* Early postoperative complications were significantly higher in group I compared to group II: (transient recurrent nerve palsy ($P < 0.04$), transient hypocalcemia ($P < 0.02$), and postoperative bleeding ($P < 0.003$)). Meanwhile permanent complications were not significantly different. VEGF-IHS revealed ten patients of group I (62.5%) to have positive staining compared to only 3 (15%) of group II. All patients of group III stained positive. The expression of VEGF was more intense in group I as compared to group II ($P < 0.02$). However, no significant difference of MVD among all groups was recorded. *Conclusion* Preoperative LI treatment may be associated with increased early postoperative morbidity and did not affect thyroid vascularity.

Keywords Graves' disease · VEGF · Lugol's Iodine · Angiogenesis · Morbidity · Immunochemistry

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Introduction

The main indications of surgical treatment in Graves' disease (GD) are recurrence of the disease after antithyroid drugs (ATD) or severe adverse reactions to ATD, large goiters, nodularity with suspicion of malignancy, sever endocrine ophthalmopathy as well as counter indication or refusal of radioiodine therapy [1–3]. Although the incidence of postthyroidectomy complications is low if performed by experienced surgeons, thyroidectomy for GD is still associated with higher morbidity rate when compared to other benign thyroid disorders [3].

Preoperative preparation of patients with hyperthyroidism is crucial to avoid more severe thyrotoxicosis resulting from leakage of thyroid hormone to the circulation at the time of surgery and to reduce intraoperative and postoperative complications related to anesthesia or surgery. Thus, the mainstay of preoperative preparation in GD is to achieve a clinical euthyroid state. Conventionally, this can be accomplished by the administration of thionamides but usually requires months to render patients euthyroid [4–7].

A far more rapid and safer control of thyrotoxicosis can be achieved by the oral administration of Lugol's iodine (LI) in combination with thionamides and ß-blockers. Administered orally for 3–10 days prior to surgery, LI prevents the further synthesis of thyroid hormone by an inhibiting effect on the Na/I-symporter. Another important action of LI is to decrease the vascularization and concomitantly to increase the consistency of the thyroid tissue [8]. The majority of endocrine surgeons administer Lugol's solution to decrease thyroid vascularity, but the available studies are mostly based on empirical or indirect evaluations such as the impression of the surgeon, making an objective assessment of the effects of LI on the gland difficult to achieve [8–11].

Angiogenesis is the development of new blood vessels from pre-existing vessels and whilst being a crucial process in normal physiology [12], it is an important pathogenic process in both benign and malignant diseases. Microvessel density (MVD) measurement has been shown to be a quantitative method of assessing angiogenesis in malignant as well as benign disorders. In tumors, MVD has been shown to correlate with the concentration and expression of proangiogenic growth factors, e.g., vascular endothelial growth factor (VEGF) and with tumor behavior [13]. VEGF is a potent stimulator of the endothelial cell growth and can stimulate both the physiological and pathological angiogenesis [14]. Increased VEGF expression has been linked with poor outcome and increased risk of metastasis or recurrence in thyroid cancer. Several studies have demonstrated the expression of VEGF in benign and malignant thyroid tissues, both in vivo and in vitro [15–18].

Angiogenesis plays an important role in goiter development with endothelial cell proliferation occurring before increased proliferation of the thyroid follicular cells [19]. Moreover, a recent study showed that the expression of VEGF was lower in autoimmune diseases like GD and Hashimoto thyroiditis, compared to autonomous growth processes, while no clear relationship between MVD and thyroid pathology could be established [20]. Jebreel et al. [21] described a significant increase in serum VEGF levels in patients with untreated GD and Hashimoto thyroiditis compared with those in healthy subjects. The aims of current clinico-pathological trial are to evaluate VEGF expression and MVD in patients with GD who received LI treatment preoperatively and to validate the hypothesis that LI treatment reduces thyroid vascularity and postthyroidectomy complications.

Material and methods

Clinical data acquisition

A cross-sectional study was performed on a consecutive series of patients with GD operated between January 1987 and January 2006 in the Department of Surgery of the Philipps University, Marburg. All patients were identified via the central electronic patient registry.

One hundred and seventy-one patients were operated on for GD. In 153/171 patients (89%), complete data sets could be obtained. There were 123 women with a median age of 36 years (range 10–75) and 30 men with a median age of 33 years (range 22–65). Length of follow-up was median 96 months postoperatively (range 12–216 months). Of 153, 42(27.5%) patients failed to be contacted for the last follow-up. Therefore, the patient's general practitioner was contacted and asked the same set of questions to obtain the relevant data.

Data related to diagnosis and treatment of GD prior to admission, as well as early postoperative outcome, were collected from the medical files (retrospective data acquisition). A structured telephone interview was conducted to assess long-term outcomes.

Postoperative complications, specifically postoperative bleeding, transient and permanent hypocalcemia, as well as transient and permanent recurrent laryngeal nerve (RLN) palsy were defined as primary endpoints. For the purpose of this study, hypoparathyrodism was assumed in any patient requiring calcium and/or vitamin D supplementation because of clinical signs of hypocalcemia. Permanent hypocalcemia was assumed if the treatment was necessary for more than six months. PTH-levels were not routinely measured.

Preoperative antithyroid treatment

According to clinical (tachycardia >90 bpm, tremor and thrill of large goiter) and biochemical criteria (TSH < 0.01 U/l, and increased FT3), 16 patients (group I) were assessed as severe thyrotoxic, and were prepared with a combination of LI (3–6 drops/day) with thionamides and ß-blockers for 3–12 days (median 6 days). The achievement of euthyroid state was defined in terms of stable heart frequency (<80 bpm) and disappearance of tremor and thrill of the goiter. The remaining 137 patients (group II) were clinically and biochemically euthyroid at the time of admission, after conventional long-term thionamides therapy.

Patients' characteristics (age, gender, type of resection) were similar in both groups. The indication for surgery was either unsuccessful treatment with antithyroid drug therapy due to noncompliance, moderate to severe ophthalmopathy, refusal of 131-Iodine therapy, and/or very large goiters.

In both groups, surgery consisted of subtotal, near-total (Dunhill), or total thyroidectomy under general anesthesia, according to a standardized operative technique, with primary identification of the RLNs and parathyroid glands, as well as neuromonitoring.

Seven stochastic paraffin blocks of patients with nontoxic goiter (group III) were used as negative control for the immunohistochemistry (IHC) analysis. Clinical data of these patients were not acquired.

IHC methods for VEGF CD31

IHC staining for VEGF was performed on naive preparations and on 4-μm sections of operative specimens. In brief, after melting and incubation in citric acid buffer for

18 h at 60°C, slides were deparaffinized and rehydrated in graded alcohol. After blocking endogenous peroxidases and unspecific binding, incubation with primary antibody (polyclonal mouse anti-VEGF antibody; 1:1,000; sc152, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was carried out overnight at 4°C and additionally enhanced using the streptavidin-based Link&Label Detection System (BioGenex, Munich, Germany). Slides were stained with diaminobenzidine-chromogen (Dako-Chemicals, Hamburg, Germany), counterstained with hematoxylin according to Mayer and mounted. Naive preparations were treated similarly without deparaffination.

CD31 immunohistochemical staining (IHS) of was performed using a polyclonal anti-CD31 AB (MEC13,3, Pharmingen, Heidelberg, Germany), essentially following the procedure described in detail elsewhere [22].

Morphometry of VEGF expression and vascularization

Expression of VEGF was then quantitated by analyzing the intensity and distribution of stained cells in ten random areas within the maximal tissue cell load, calibrated against an internal standard (Leica Q500 MC/MPS60 Digital Image Processing & Analysis System, Wetzlar, Germany). Values represented the area of unequivocally stained cells per high power field (HPF, 160×).

For quantitation of angiogenesis, MVD was morphometrically determined for each tissue sample using two established protocols. Assessment of MVD was done by stereological assessment of contiguous tissue sections, analyzing the number of vessels and the area they cover, as well as the relational areas of cells and stroma [23]. The method allows a precise calculation of the area of micro vessels in a given tissue volume, hence the term MVD (n/mm^2). Twenty random areas were analyzed for each section (Figs. 1 and 2).

Statistical analysis

Estimated values of VEGF and MSD as well as clinical data were implemented in SPSS 12.0® (SPSS, Chicago, IL, USA). Univariable analysis (cross tables, Mann–Whitney *U*-test and *t*-test) was used to determine associations between morbidity and type of preoperative treatment. Probabilities of less than 0.05 were accepted to identify significance.

Results

The level of TSH-receptor antibodies (TRAb) was preoperatively measured in 87/153 patients (56.8%) and was found increased in 80/87 (92%). In seven patients (8%) the TSH-R-Ab level was within the normal range. In these patients' clinical symptoms, measurement of endocrine orbitopathy and ultrasonography of the thyroid gland confirmed the suspected GD.

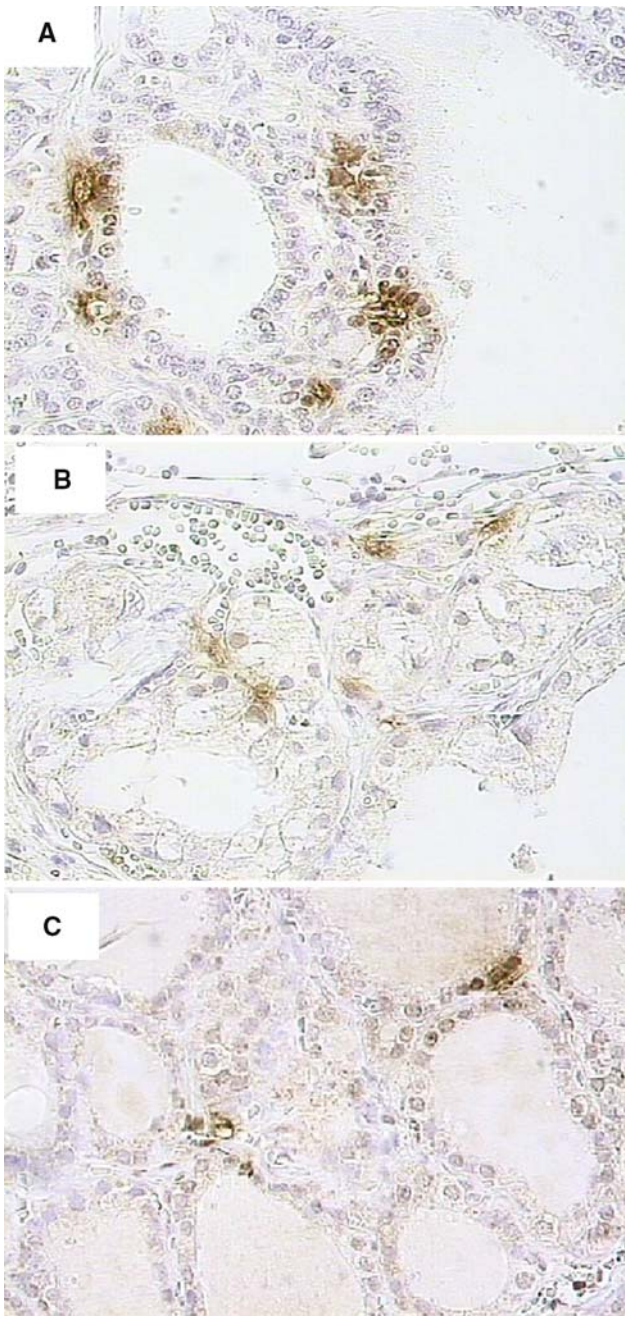

Fig. 1 Immunohistochemistry for VEGF (Antibody-dilution 1:1,000) in the cytoplasm of follicular cells in thyroid tissue of all groups. (**a**) Lugol treatment Graves thyroid, strongly positive for VEGF, (**b**) non-Lugol treatment Graves thyroid, and (**c**) non-Graves multinodular thyroid (400×)

All operations were performed or supervised by surgeons with special expertise in thyroid and parathyroid surgery. No postoperative mortality was recorded. There

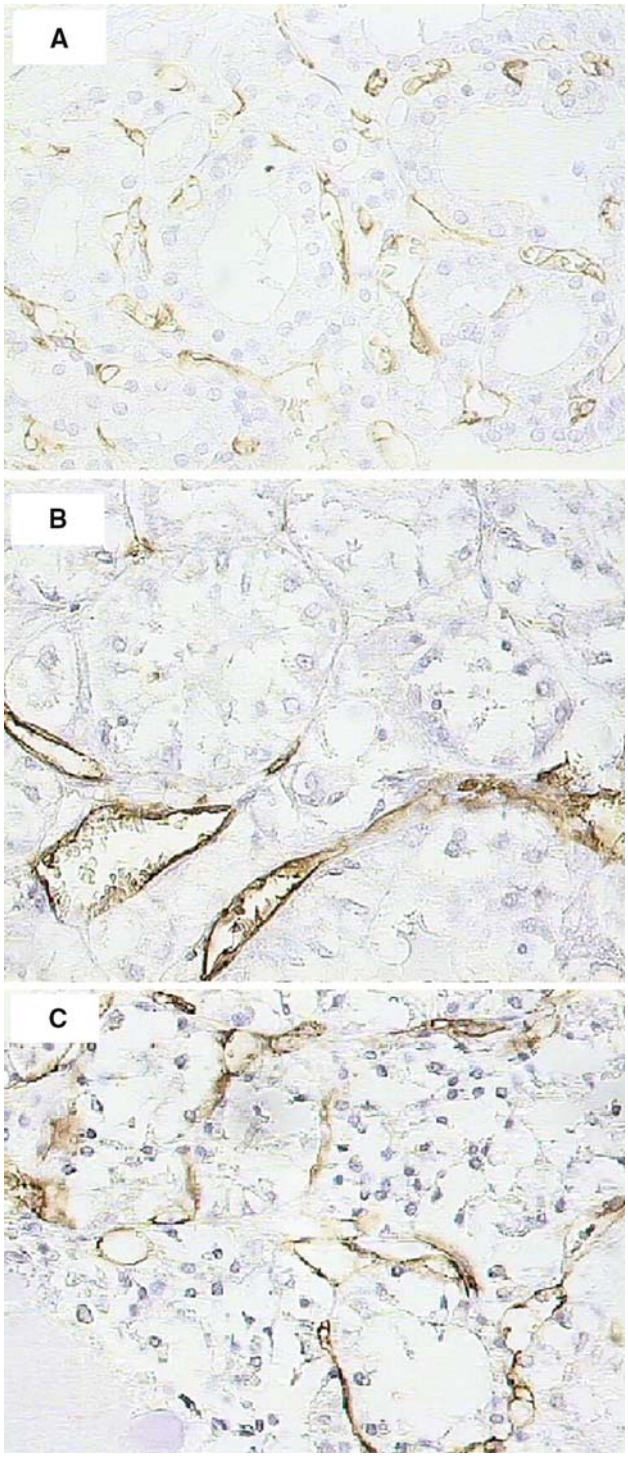

Fig. 2 Positive immunoreactivity of endothelial cells between thyroid follicular structures in (**a**) Lugol treatment Graves thyroid, (**b**), non-Lugol treatment Graves thyroid, and (**c**) non-Graves multinodular thyroid (anti-CD-31, 400×)

were no perioperative thyrotoxic crises in either of the groups. Transient hypocalcemia occurred in 46% of the patients in group I, whereas only in 19.2% of the patients in group II ($P = 0.026$). Permanent hypocalcemia occurred in 15.3% in group I, compared to only ten cases (7.1%) in group II ($P = 0.265$). Postoperative bleeding required intervention in three patients (18.7%) of group I compared to only one patient (0.7%) in group II ($P < 0.003$). Transient RLN palsy occurred in 23% of the cases in the first group I and in 7.1% of group II ($P = 0.042$). Only one case of permanent RLN palsy was registered in each group ($P = 0.475$) (Table 1).

The weight of the resection specimens in group I was, with a median of 83 g (range 35–213 g), higher than in group II with a median of 57 g (range 7–275 g), without reaching statistical significance ($P = 0.07$). In each group goiter volume was not associated with postoperative morbidity. Finally, patients in group I had a longer hospital stay (median of 6 days; range 3–15) than patients of group II (median of 4 days, range 1–18) ($P = 0.005$).

The immunohistochemical analysis showed that ten patients from group I (77%) expressed positive VEGF staining compared to only three patients (15%) in group II ($P = 0.002$) (Table 2). Furthermore, all patients in group III expressed positive VEGF ($P = 0.0001$) (Fig. 1). Morphometrical analysis showed no difference in VEGF score in the positive cases of groups I and III ($P = 0.280$). VEGF correlates only in group I with the MSD ($P = 0.041$) (Fig. 3). The mean values of MSD in all groups were not significantly different.

Discussion

GD is the most common causes of hyperthyroidism [24, 25]. Hyperthyroidism is associated with important hemodynamic changes, including high output state, increased heart rate, and cardiac contractility, as well as decreased peripheral resistance that are related to both direct cardiostimulatory effects of thyroid hormone and increased peripheral oxygen consumption [5, 25]. Henry Plummer demonstrated in 1923 that the preoperative administration of inorganic iodide would prevent the life threatening intraoperative bleedings and thyrotoxic crises, though dramatically reducing the mortality of surgery in exophtalmic goiter [26]. His work was hailed as one of medicines greatest gifts to surgery and the Germans coined the fitting term for it "*Plummerung*" [27].

It is a common place that thyroid enlargement and hyperfunction in GD are accompanied by a markedly increased blood flow, responsible for the technical difficulties during thyroidectomy and increased postoperative morbidity. About 80 years after the pioneer work of Henry Plummer, the mechanisms mediating thyroid vascular modification remain not entirely clear, although immunohistochemical analyses showed an increased expression of VEGF and vascular density in GD specimens compared to

Table 1 Postoperative morbidity

	Bleeding	Temporary paresis	Permanent palsy	Temporary hypocalcemia	Permanent hypocalcemia
Total ($n = 153$)	4 (2.6%)	13 (8.5%)	2 (1.3%)	33 (21.6%)	12 (7.8%)
Group I ($n = 16$)	3* (18.7%)	4** (25%)	0	7*** (43.7%)	2 (12.5%)
Group II ($n = 137$)	1 (0.8%)	9 (6.5%)	2 (1.5%)	26 (18.9%)	10 (7.3%)

Comparison between groups I and II using the Mann–Whitney *U*-test; * $P = 0.042$, ** $P = 0.003$, *** $P = 0.026$

Table 2 Results of morphometrical analysis of IHS for VEGF and MVD (n/mm^2)

	VEGF-positive	MVD (mean)
Group I ($n = 16$)	10/16* (62.5%)	21.7 (SD 5.57)
Group II ($n = 20$)	3/20 (15%)	21.8 (SD 3.84)
Group III ($n = 7$)	7** (100%)	24.04 (SD 6.73)

* $P = 0.002$ for comparison between groups I and II and ** $P = 0.001$ when group III was compared to the two other groups

SD, standard deviation

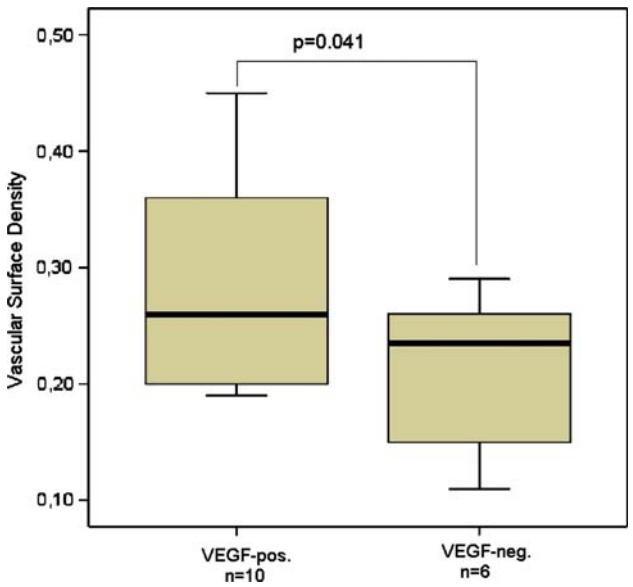

Fig. 3 Box and whisker plot (median = bar, interquartile range = box and whiskers = standard deviation) of MVD-score (n/mm^2), in correlation to the status of VEGF expression in the Lugol treated Graves-Patients assessed by the morphometric analysis using the Leica computer system

normal thyroid tissue [21]. A study by Yamada et al. [28] has demonstrated that iodide at high concentration decreases the expression of VEGF in cultured Human thyroid follicles.

In the current study, VEGF was expressed in 62.5% of the "plummered" patients, 15% of the GD-patients treated with ATD only and in the seven stochastic goiter patients. Furthermore, there was not only an increase in the distribution but also in the intensity of VEGF immunopositivity in the "plummered" GD-patients in comparison with GD-patients treated with ATD only. However, this may indicate an effect of LI on the expression of VEGF as others suggested [21].

It is currently believed that angiogenesis is controlled by a balance between angiogenic stimulators and inhibitors, rather than by the activity of a single regulator. In addition to angiogenic inducers, a number of potential antiangiogenic factors have been identified, including thrombospondin-1, angiostatin, and endostatin [29].

Among our series, regardless of treatment, MVD was similar in all groups, although there were differences in group I relative to VEGF positivity. It is possible that other factors that regulate the angiogenic phenotype were expressed, in terms of stimulating angiogenesis, as well as mechanisms involving loss of inhibitors.

Doppler techniques seem to be a good method to evaluate blood flow in the thyroid gland. In a recent study, Nagasaki et al. [6] proved the intense blood flow of hyperthyroid tissue, measured in Doppler mode at the inferior thyroid artery but that did not correlate significantly with VEGF expression.

Besides reducing the circulating levels of thyroid hormones, LI treatment also decreases the vascularity of the thyroid tissue. Iitaka et al. [22] observed that intrathyroid blood flow assessed by Doppler ultrasound examination was significantly lower after ATD treatment, as well as the VEGF expression, but the exact mechanism of action is not well understood.

The decrease of thyroid vascularisation following "Plummerung" was proved by a number of authors using Doppler ultrasound examination, but no patent explanation was granted, if this reduction was based on decrease of MVD [7, 27, 30]. Currently, there is only one study evaluating the effect of LI on patients with GD using MVD but without a correlation to postoperative morbidity [31]. According to this study, after LI treatment in 17 GD-patients, who were not thyrotoxic, MVD as determined by IHC and CD-34 expression, were significantly lower than values obtained in the 19 GD-control patients without LI treatment.

Despite the limitation of the retrospective design of the current study, to the best of our knowledge, this is the first study to demonstrate the effect of iodide solution on postoperative morbidity and VEGF expression. Only 15%

of the patients preoperatively treated with ATD expressed positive VEGF staining, compared to 62.5% of the "plummered" cases ($P = 0{,}002$). These results demonstrate that achieving an euthyroid state after administration of ATD alone without LI correlates with a significant reduction of VEGF expression. However, no significant difference was observed between the two groups, in terms of vascular density. Furthermore, the VEGF down-regulation correlated with a significant lower postoperative morbidity of the patients conventionally prepared with ATD, in terms of postoperative bleeding ($P < 0.003$), transient RLN paresis ($P < 0.04$), and transient hypoparathyroidism ($P < 0.02$).

Conclusion

Although VEGF correlated with the increased vascularity of GD, vascular density was not affected by LI treatment. The necessity of further studies on the impact of LI on VEGF-mediated vascular permeability and blood flow is suggested.

Patients with thyrotoxic Grave's disease who were preoperatively treated with Lugol's solution have an increased postoperative morbidity, most likely as a reflection of the severity of the toxic state. However, thyroidectomy in thyrotoxic Grave's cases should be reserved for specialized centers, particularly, when surgical treatment of the disease cannot be delayed.

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