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Aula Magna "C. Golgi"
& WEBINAR

**L' elettroporazione nel trattamento delle patologie
malformative linfatiche e vascolari in
otorinolaringoiatria**

Prof. Marco Benazzo

*Direttore U.O.C di Otorinolaringoiatria
Fondazione IRCCS Policlinico San Matteo*



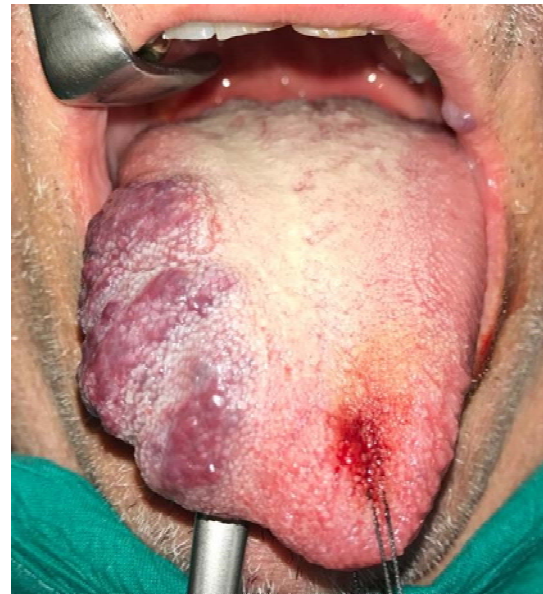
CLINICAL CASE

CASE 1

Male, 68 years old

Cavernous hemangioma of the right lingual edge (38 x 10 x 15 mm)

Symptomatic, bleeding lesion



Pre-operative

CLINICAL CASE

PROCEDURE

- Intralesional injection of 2 mg of bleomycin
- Electroporation of the lesion with electrode N-20-HG, total of 7 applications

OUTCOME

- Mild dysphagia and odynophagia for 2 months
- NED 1 year after the treatment

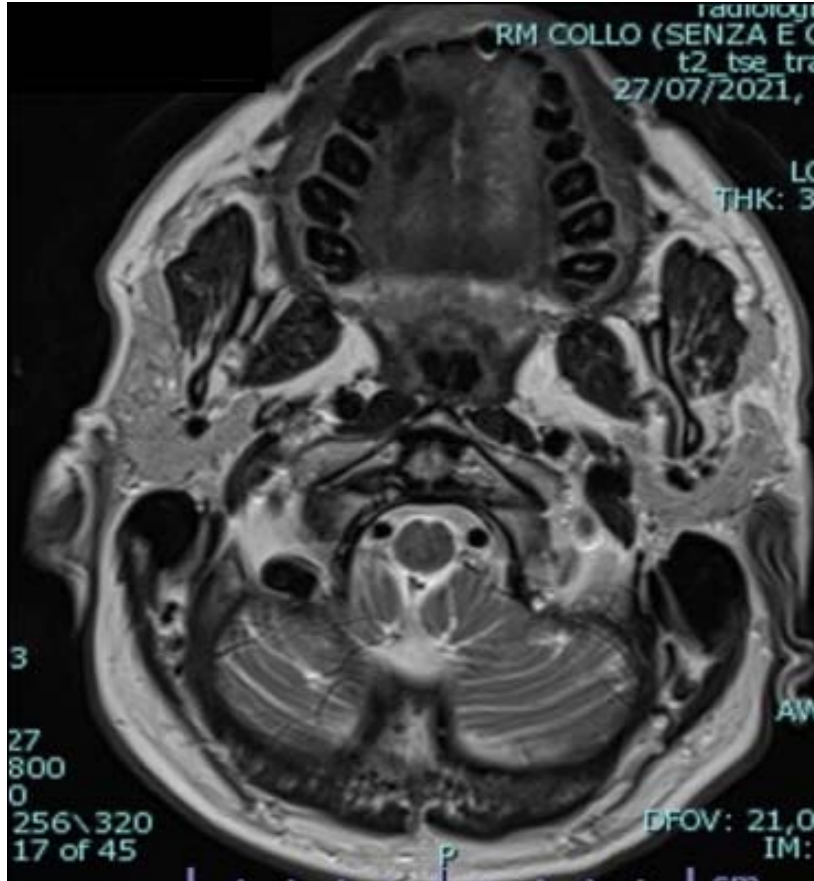


Immediately after the treatment
(vascular lock effect)



1 month after the treatment

CLINICAL CASE

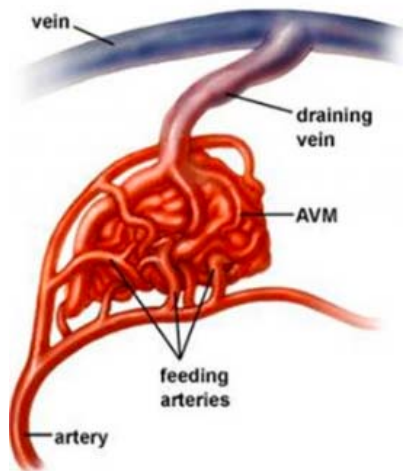


1 year after the operation
NED

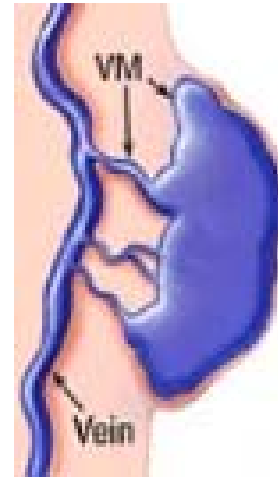
INTRODUCTION

VASCULAR MALFORMATIONS

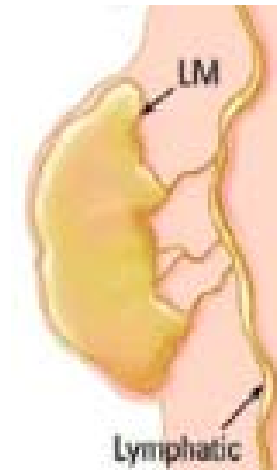
Arterious (AMs, high flow)



Venous (VMs, low flow)



Lymphatic (LMs, low flow)



Vascular malformations in the head and neck area account for 40% of all vascular malformations cases

Treatment is necessary in the presence of clinical symptoms, personal discomfort, cosmetic disturbance

Greene AK. Vascular anomalies: Current overview of the field. Clin Plast Surg 2011

INTRODUCTION

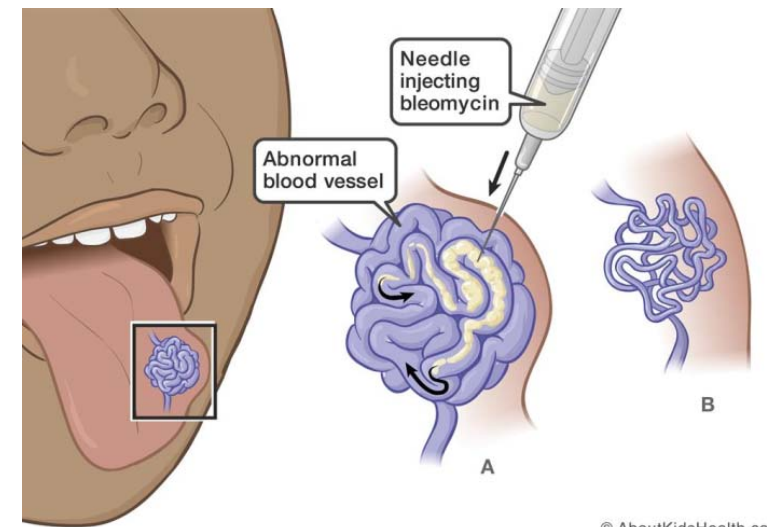
TREATMENT OF VASCULAR MALFORMATIONS

SURGERY

- ✓ Surgical resection
- ✓ High recurrence rates, especially for VMs and LMs

SCLEROTHERAPY

- ✓ Intralesional injection of sclerosing agents (Polidocanol, Bleomycin, Picibanil)
- ✓ First-line treatment for VMs and LMs
- ✓ Success rate is in the range of 71–100%



Mahady K, et al., “Vascular anomalies of the head and neck in children”. *Quant Imaging Med Surg*. 2015

Horbach S.E.R., et al., “Sclerotherapy for low-flow vascular malformations of the head and neck: A systematic review of sclerosing agents” A systematic review. *J Plast Reconstr Aesthet Surg*. 2016

Zeevi I, et al., “Sclerotherapy of Vascular Malformations in the Oral Cavity-Minimizing Postoperative Morbidity”. *Medicina (Kaunas)*. 2020

Kostusiak M, et al. “Bleomycin Electrosclerotherapy Treatment in the Management of Vascular Malformations”. *Dermatol Surg*. 2022

INTRODUCTION

SCLEROTHERAPY



ELSEVIER

REVIEW

Sclerotherapy for low-flow vascular malformations of the head and neck: A systematic review of sclerosing agents[☆]

Sophie E.R. Horbach^{a,d,*}, Max M. Lokhorst^{a,b,d},
Peerooz Saeed^b,
Claire M.F. de Gouyon Matignon de Pontouraude^b,
Aniki Rothová^c, Chantal M.A.M. van der Horst^a

^a Department of Plastic, Reconstructive and Hand Surgery, Academic Medical Center, Amsterdam, The Netherlands

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Received 17 September 2015; accepted 30 October 2015

Results: The literature search yielded 1155 articles, among which 36 (1552 patients) were included in the systematic review. The quality of evidence was low. Pingyangmycin, absolute ethanol, OK-432, ethanolamine oleate, bleomycin, polidocanol, doxycycline, and sodium tetradecyl sulfate (STS) were the most reported sclerosing agents. All agents seem effective, and the mean overall response varies from 71% to 100%. Complications occurred more frequently after ethanol sclerotherapy (18%), compared to other sclerosing agents (0–6%). Cellulitis and ulceration were encountered following sclerotherapy with most sclerosing agents, but skin necrosis was particularly observed after ethanol. Facial nerve paralysis occurred only after OK-432 (0.05%) and ethanol sclerotherapy (6%).

Conclusions: This systematic review could not identify a significantly superior sclerosing agent in terms of effectiveness, due to the low quality of the available evidence. Until stronger evidence is available, the difference in complication rates is potentially the deciding factor in the choice between sclerosing agents. As a significantly higher complication rate and more severe local complications were encountered after using absolute ethanol, we cannot recommend this agent for sclerotherapy of cervicofacial vascular malformations.

INTRODUCTION

SCLEROTHERAPY






Journal of
Clinical Medicine



Article

Bleomycin for Percutaneous Sclerotherapy of Venous and Lymphatic Malformations: A Retrospective Study of Safety, Efficacy and Mid-Term Outcomes in 26 Patients

Franck Nevesny¹, Olivier Chevallier¹, Nicolas Falvo¹, Kévin Guillen¹, Alexandre Malakhia¹, Julie Pellegrinelli¹, Pierre-Olivier Comby², Bertille Bonniaud³, Marco Midulla¹ and Romaric Loffroy^{1,*}

[J Clin Med.](#) 2021 Mar; 10(6): 1302.

5. Conclusions

Although our study included a limited number of patients, it suggests that percutaneous bleomycin is safe and effective for the treatment of VMs and LMs. The lesion size reduction was 50% or more for 64% of the VMs and 87% of the LMs. Most patients reported improvements in pain/discomfort and cosmesis, although only a minority felt markedly improved. The patient-reported outcomes did not consistently reflect the efficacy in terms of lesion size reduction. Finally, further research is needed to understand why bleomycin sclerotherapy was more effective in the LM group than in the VM group.

INTRODUCTION

ELECTROCHEMOTHERAPY (ECT)

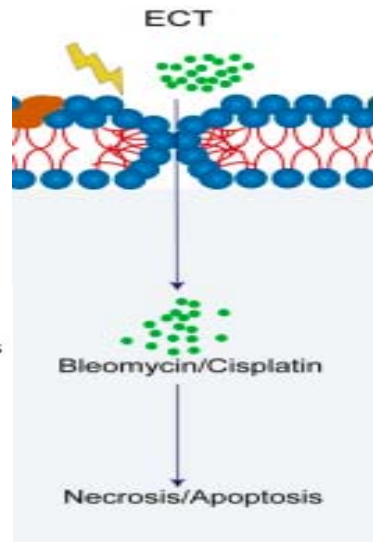
Local therapeutic approach for the treatment of tumours nodules which are independent of histology

Anticancer Drug Injection (Bleomycin)

+

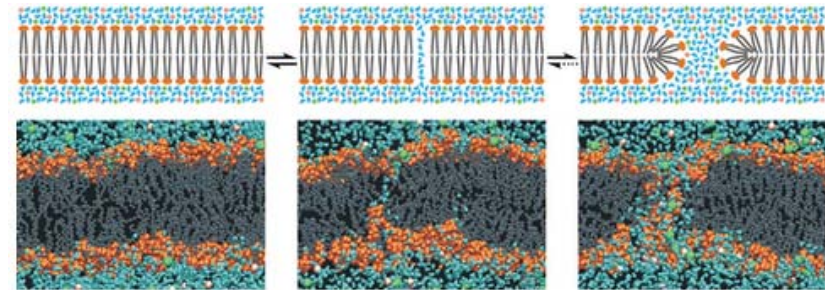
Reversible Electroporation
(physical phenomenon)

- Cytotoxicity
- Sclerosing agent



- 1) i.t. or i.v. injection of cytotoxic
- 2) Insertion of needle electrodes in tumor
- 3) Pulse application
- 4) repeat steps 2 and 3 for larger or multiple tumors

- Increase cell permeability
- Vasoconstriction reflex
- Local activation of immune system cells

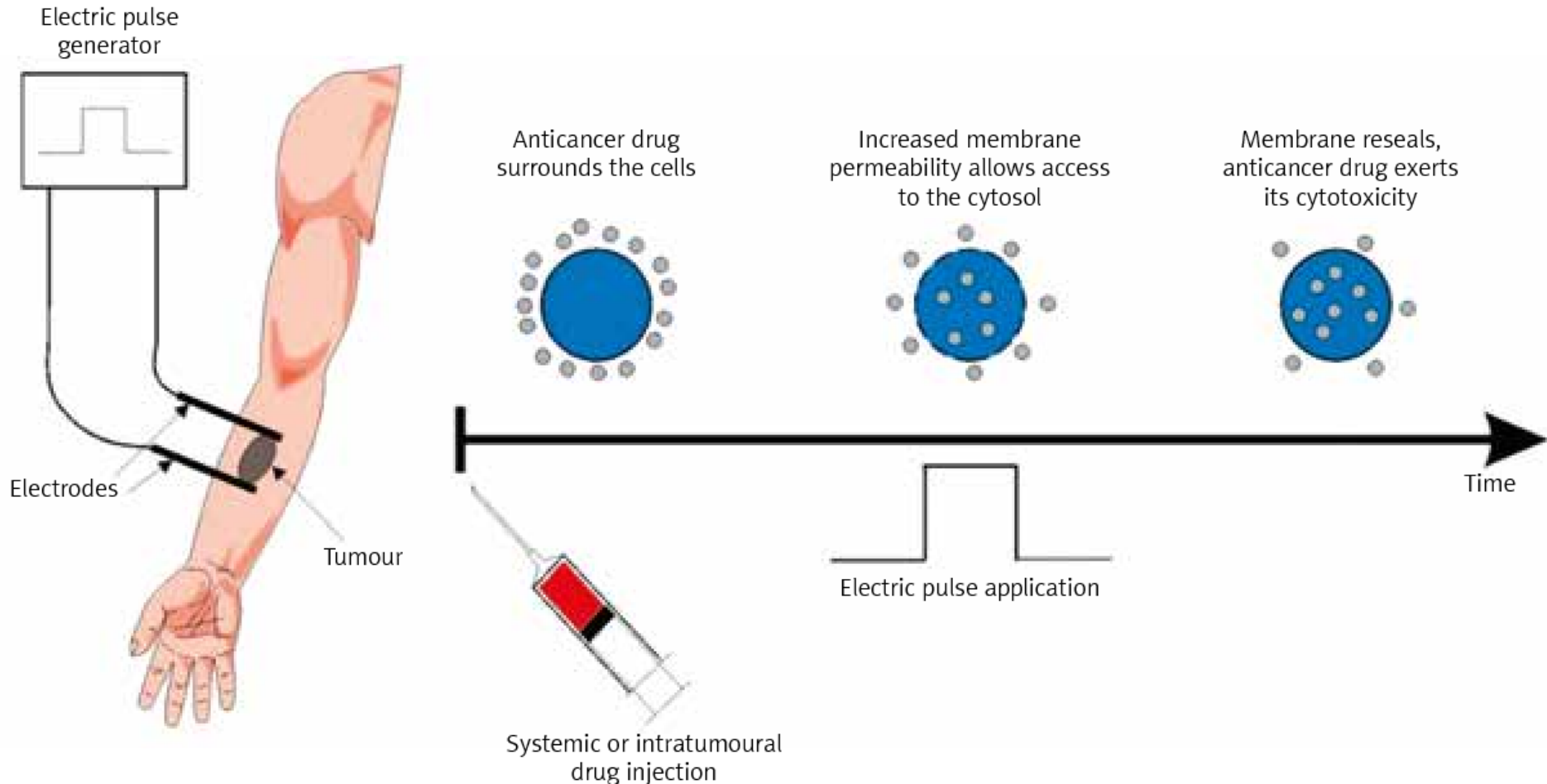


Yarmush ML, et al. 2014.
Annu. Rev. Biomed. Eng. 16:295-320

Gong X, et al. "Advances of Electroporation-Related Therapies and the Synergy with Immunotherapy in Cancer Treatment." *Vaccines (Basel)*. 2022

INTRODUCTION

ELECTROCHEMOTHERAPY (ECT)

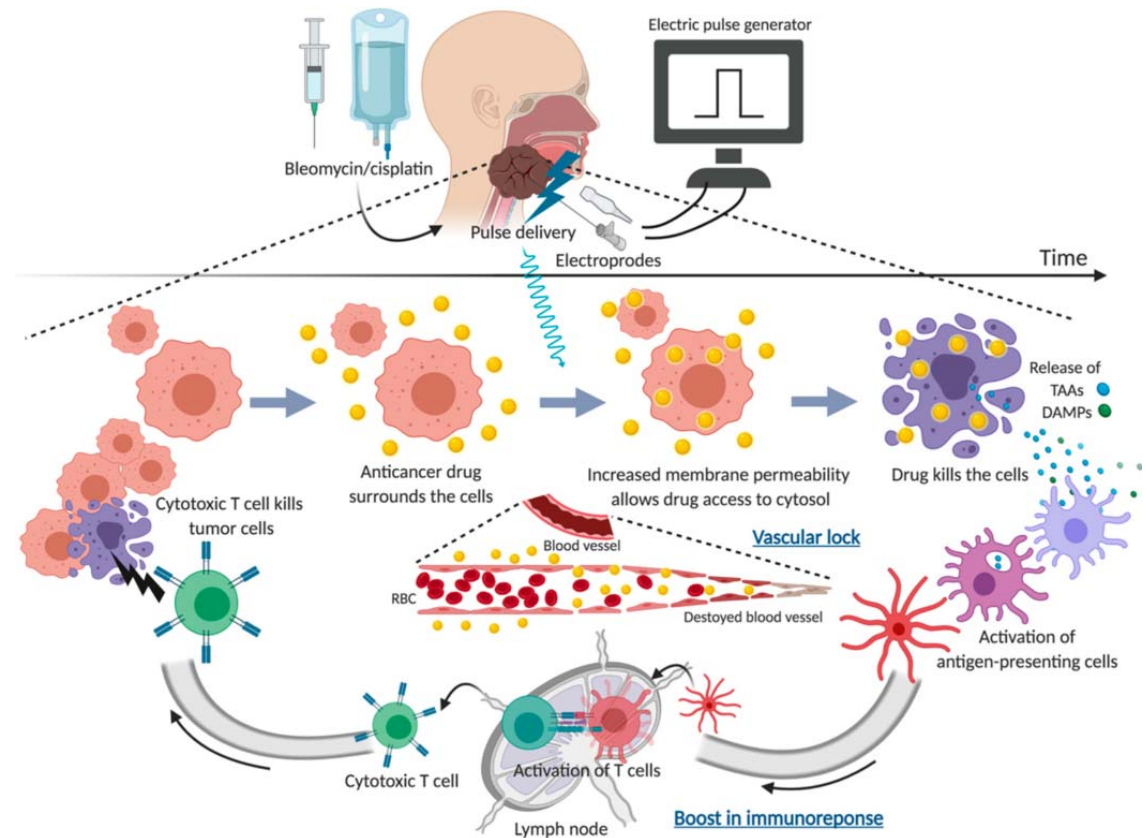


Bleomycin's toxicity increases up to 10 times through cell membranes electroporation in vivo

INTRODUCTION

ELECTROCHEMOTHERAPY (ECT)

- ✓ Tumoral cells die for apoptosis during the mitotic division
- ✓ Selective cellular death of the neoplastic cell with high mitotic index
- ✓ Usually tumoral nodes disappear within 30-60 days
- ✓ Non thermal ablative method, healthy tissue is spared
- ✓ Boost in immunoreponse



Enokida, T., et al., "Electrochemotherapy in the Treatment of Head and Neck Cancer: Current Conditions and Future Directions". *Cancers* 2021

INTRODUCTION

ELECTROCHEMOTHERAPY (ECT)

Cliniporator VITAE



INTRAVENOUS

Bleomycin Standard Dose: 15000 IU/m²

Electric Impulses are applied 8 min after drug administration in order to allow capillary diffusion

Time window of electric impulses application is 20 min

INTRATUMORAL

Volume $ab^2\pi/6$	$D < 0.5 \text{ cm}^3$	$0.5 \text{ cm}^3 < D < 1 \text{ cm}^3$	$D > 1 \text{ cm}^3$
BLM - 1000IU/ml	1ml/cm ³ (> 0,1ml)	0.5ml/cm ³	0,25 ml/cm ³
CDDP - 2mg/ml	1ml (2 mg)/cm ³	0.5ml (1 mg)/cm ³	0,25 ml (0.5mg)/cm ³

Mir L. Electrochemotherapy. EJC Suppl 2006

INTRODUCTION

ELECTRODES



Plate



Hexagonal



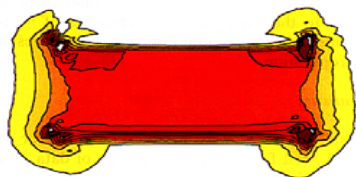
Finger L.



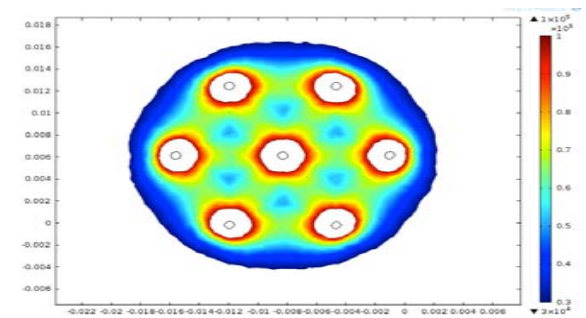
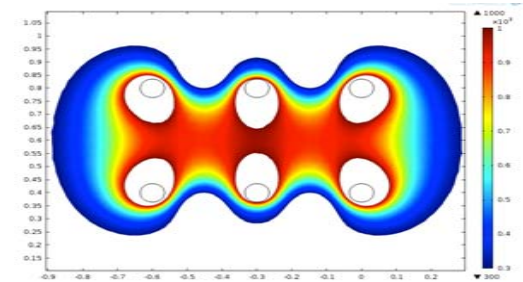
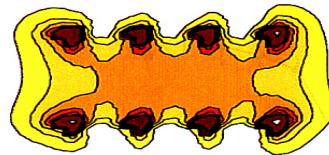
Finger O.



Linear



2 mm

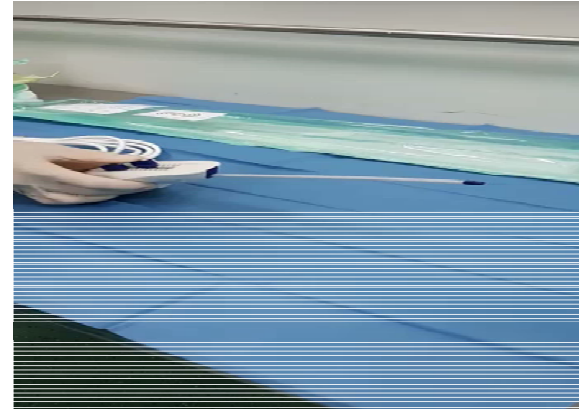


INTRODUCTION

ELECTRODES



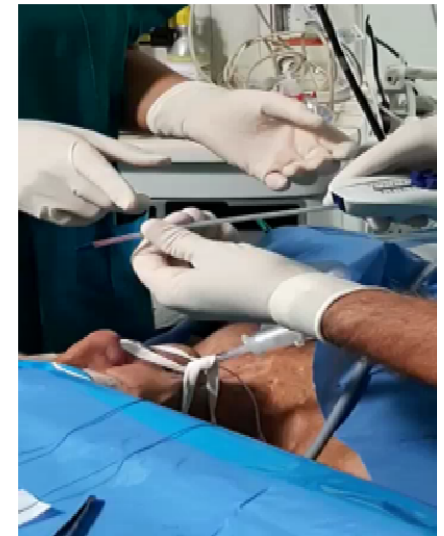
Adjustable Hexagonal



Stinger



Adjustable Linear

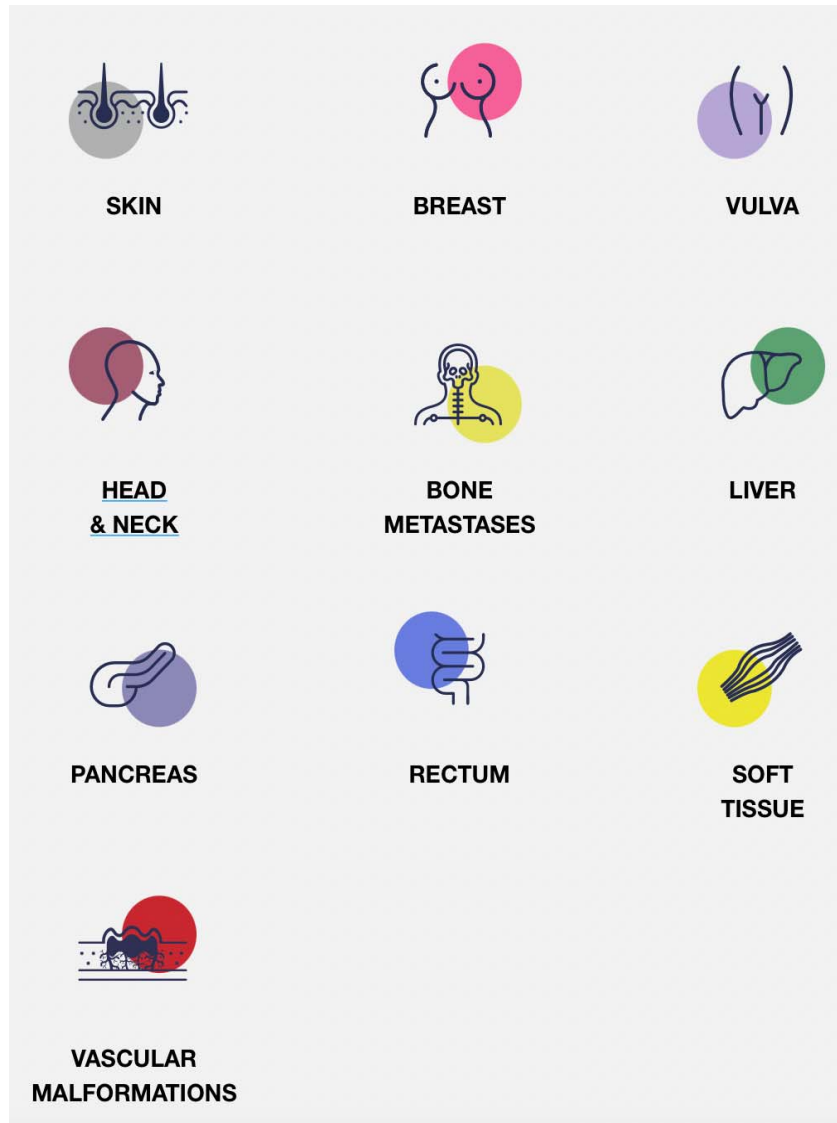


Variable geometry

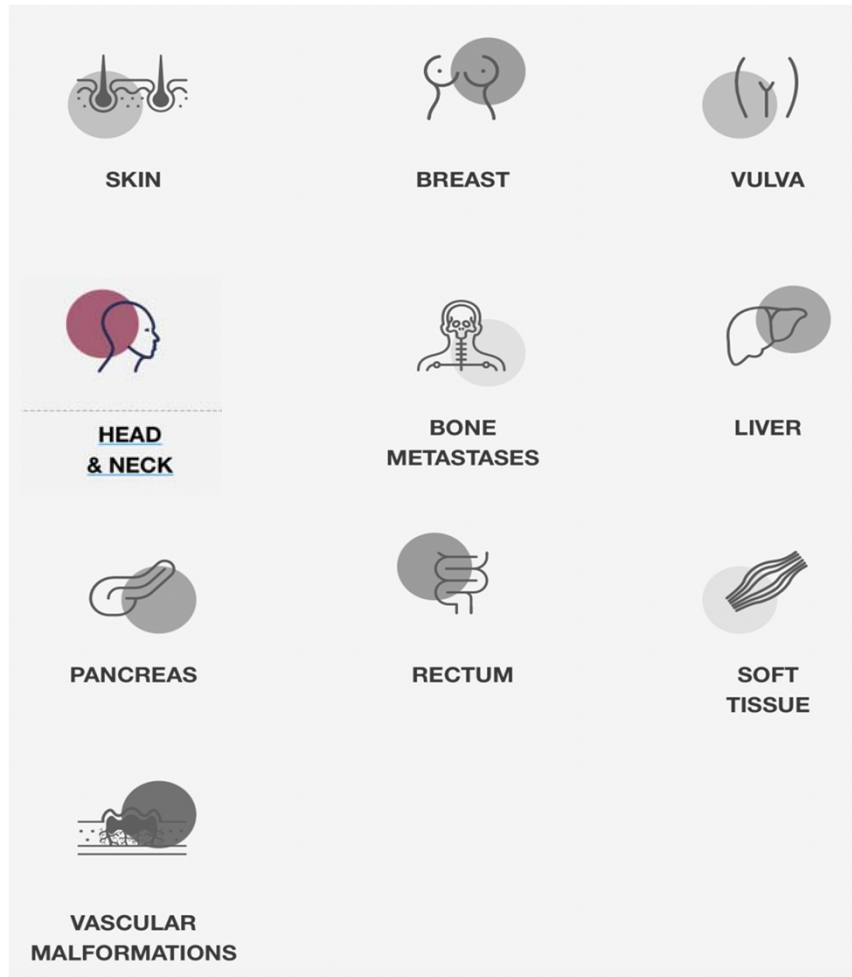


INTRODUCTION

Electrochemotherapy can effectively treat tumours located in:



INTRODUCTION



Review

Electroporation in Head-and-Neck Cancer: An Innovative Approach with Immunotherapy and Nanotechnology Combination

Silvia Pisani ^{1,*}, Giulia Bertino ¹, Adriele Prina-Mello ^{2,3}, Laura Deborah Locati ^{4,5}, Simone Mauramati ¹, Ida Genta ⁶, Rossella Dorati ⁶, Bice Conti ⁶ and Marco Benazzo ^{1,7}

- ¹ Department of Otorhinolaryngology, Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi, 19, 27100 Pavia, Italy
 - ² LBCAM, Department of Clinical Medicine, Trinity Translational Medicine Institute, Trinity College Dublin, Dublin 8, Ireland
 - ³ Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, D02 W085 Dublin, Ireland
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 - ⁵ Department of Internal Medicine and Therapeutics, University of Pavia, 27100 Pavia, Italy
 - ⁶ Department of Drug Sciences, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy
 - ⁷ Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, 27100 Pavia, Italy
- * Correspondence: s.pisani@smatteo.pv.it or silvia.pisani01@universitadipavia.it

Simple Summary: This review provides a summary of the head-and-neck squamous cell carcinoma (HNSCC) biological characteristics and its current treatments. Furthermore, insight and outlook on the relationship between electroporation and its implementation (combination with nanotechnology and immunotherapy) in the treatment of H&N cancers are provided.



Citation: Pisani, S.; Bertino, G.; Prina-Mello, A.; Locati, L.D.; Mauramati, S.; Genta, I.; Dorati, R.; Conti, B.; Benazzo, M. Electroporation in Head-and-Neck Cancer: An Innovative Approach with Immunotherapy and Nanotechnology Combination. *Cancers* **2022**, *14*, 5363. <https://doi.org/10.3390/cancers14215363>

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Abstract: Squamous cell carcinoma is the most common malignancy that arises in the head-and-neck district. Traditional treatment could be insufficient in case of recurrent and/or metastatic cancers; for this reason, more selective and enhanced treatments are in evaluation in preclinical and clinical trials to increase in situ concentration of chemotherapy drugs promoting a selectively antineoplastic activity. Among all cancer treatment types (i.e., surgery, chemotherapy, radiotherapy), electroporation (EP) has emerged as a safe, less invasive, and effective approach for cancer treatment. Reversible EP, using an intensive electric stimulus (i.e., 1000 V/cm) applied for a short time (i.e., 100 μ s), determines a localized electric field that temporarily permeabilizes the tumor cell membranes while maintaining high cell viability, promoting cytoplasm cell uptake of antineoplastic agents such as bleomycin and cisplatin (electrochemotherapy), calcium (Ca^{2+} electroporation), siRNA and plasmid DNA (gene electroporation). The higher intracellular concentration of antineoplastic agents enhances the antineoplastic activity and promotes controlled tumor cell death (apoptosis). As secondary effects, localized EP (i) reduces the capillary blood flow in tumor tissue ("vascular lock"), lowering drug washout, and (ii) stimulates the immune system acting against cancer cells. After years of preclinical development, electrochemotherapy (ECT), in combination with bleomycin or cisplatin, is currently one of the most effective treatments used for cutaneous metastases and primary skin and mucosal cancers that are not amenable to surgery. To reach this clinical evidence, in vitro and in vivo models were preclinically developed for evaluating the efficacy and safety of ECT on different tumor cell lines and animal models to optimize dose and administration routes of drugs, duration, and intensity of the electric field. Improvements in reversible EP efficacy are under evaluation for HNSCC treatment, where the focus is on the development of a combination treatment between EP-enhanced nanotechnology and immunotherapy strategies.

Keywords: electroporation; electrochemotherapy; immunotherapy; nanotechnology; head-and-neck cancer

Cancers **2022**, *14*, 5363. <https://doi.org/10.3390/cancers14215363>

<https://www.mdpi.com/journal/cancers>

INTRODUCTION

SCIENTIFIC QUESTION

Can Immuno-nanotechnology improve chemotherapy performances of electrochemotherapy?

Ricerca Corrente 2022
Project form

ALLEGATO 1

PROJECT TITLE

Nano-Electro-Chemo-Immuno Therapy (NECIT) to enhance head and neck cancer treatment

RESEARCH AREAS

*(please indicate the one that **best** applies to your project, if more than one, please indicate in order of priority)*

Immunopathology, Allergy, **Immunotherapy**
Haematology
Cardiovascular Respiratory
Infection
Transplantation

This project is a proof of concept to evaluate the efficacy of Pembrolizumab functionalized Pegylated liposomes loaded with Bleomycin (PePeLiB) in combination with electroporation for the treatment of head and neck squamous cell carcinoma (HNSCC) exploiting in vitro (2D and 3D) and in vivo models.

Ricerca Corrente 2021: Nano-Electro-Chemo-Immuno Therapy (NECIT) to enhance head and neck cancer treatment (08053922) 01/01/2023-01/01/2025

INTRODUCTION

ELECTROSCLEROTHERAPY

- ✓ Electroscleroterapy is based on the same principles of ECT
- ✓ Route of administration: **intralesional only**
- ✓ Clinical indication: **vascular malformations** superficial and/or deep
- ✓ Bleomycin is also a sclerosing agent

Vascular malformations progressively reduce or disappear thanks to the sclerosing, cytostatic effect of bleomycin and the vascular lock effect of electroporation



INTRODUCTION

ELECTROSCLEROTHERAPY

Available online at www.sciencedirect.com
ScienceDirect

ELSEVIER

British Journal of Oral and Maxillofacial Surgery 55 (2017) 977–979

BRITISH
Journal of
Oral and
Maxillofacial
Surgery
www.bjoms.com

Case report
Bleomycin electrosclerotherapy: new treatment to manage vascular malformations

L. McMorrow^{a,*}, M. Shaikh^b, G. Kessell^b, T. Muir^a

^a Department of Plastic and Reconstructive Surgery, James Cook University Hospital, Middlesbrough, UK
^b Department of Anaesthesia, James Cook University Hospital, Middlesbrough, UK

Accepted 3 October 2017
Available online 18 October 2017

Journal of Vascular Surgery: Venous and Lymphatic Disorders

Volume 9, Issue 3, May 2021, Pages 731-739

ELSEVIER

Clinical research study
Venous and lymphatic malformations

Bleomycin electrosclerotherapy in therapy-resistant venous malformations of the body

Walter A. Wohlgemuth PhD^a, Rene Müller-Wille^b, Lutz Meyer^c, Moritz Wildgruber^d, Moritz Guntau^a, Susanne von der Heydt^e, Maciej Pech^f, Alessandro Zanasi^g, Lilit Flöther^h, Richard Brill^a

Conclusions

The combination of bleomycin sclerotherapy and reversible electroporation is technically feasible and associated with improvements in patients with VMs despite previous unsuccessful invasive therapy. The demonstrated VM lesion reduction and clinical effectiveness warrant a future prospective randomized trial...

INTRODUCTION

ELECTROSCLEROTHERAPY: procedure

INTRALESIONAL			
Volume $ab^2\pi/6$	$D < 0.5 \text{ cm}^3$	$0.5 \text{ cm}^3 < D < 1 \text{ cm}^3$	$D > 1 \text{ cm}^3$
BLM - 1000IU/ml	1ml/cm ³ (> 0,1ml)	0.5ml/cm ³	0,25 ml/cm ³

Electric Impulses are applied immediately after drug injection



ELECTROSCLEROTHERAPY

CLINICAL CASES

- ✓ Treatment of vascular malformation with Bleomycin Electrosclerotherapy since 2021
- ✓ Five cases : 4 adults and 1 child



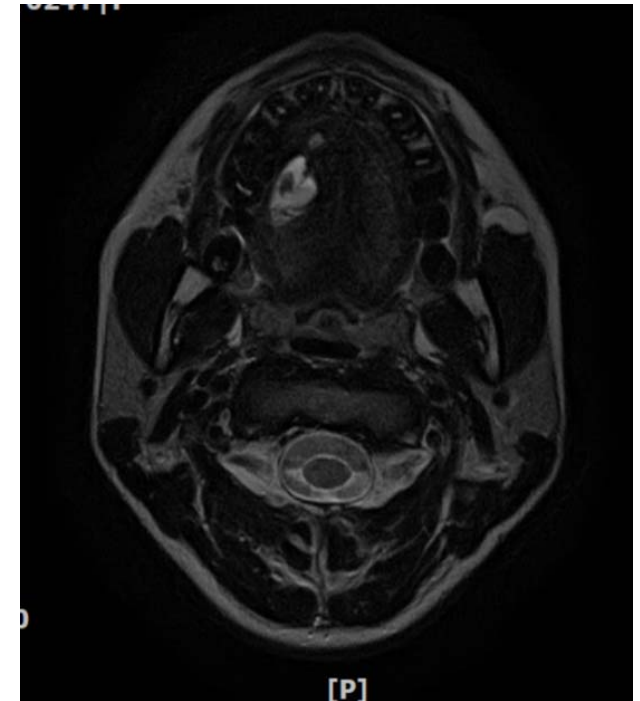
ELECTROSCLEROTHERAPY

CLINICAL CASES

Female, 24 years old

Cavernous hemangioma of the right lingual edge and submandibular

Lesions not clinically visible, but the tongue lesion was symptomatic (pain)



Pre-operative MRI

ELECTROSCLEROTHERAPY

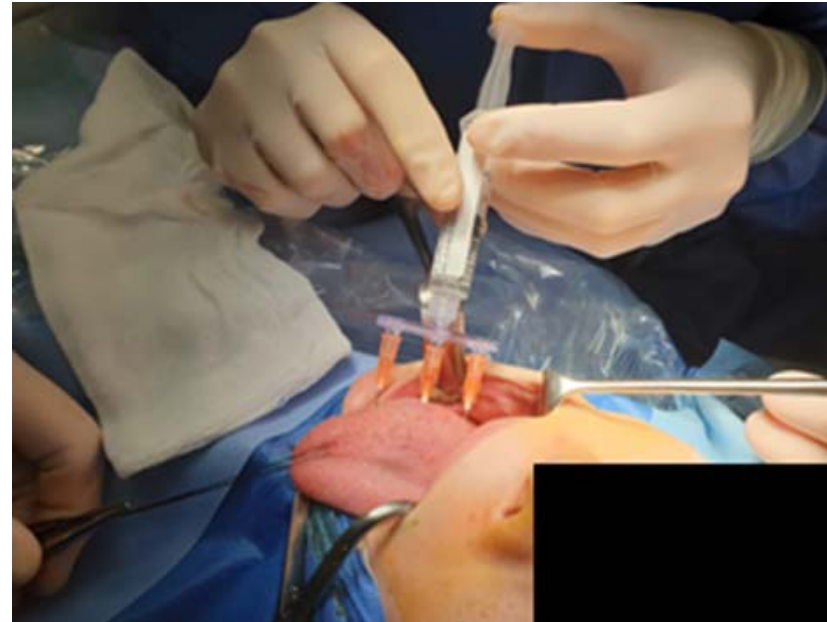
CLINICAL CASES

PROCEDURE

- Intralesional injection of 1 mg of bleomycin
- Electroporation of the lesion with electrode N-30-HG, total of 2 applications

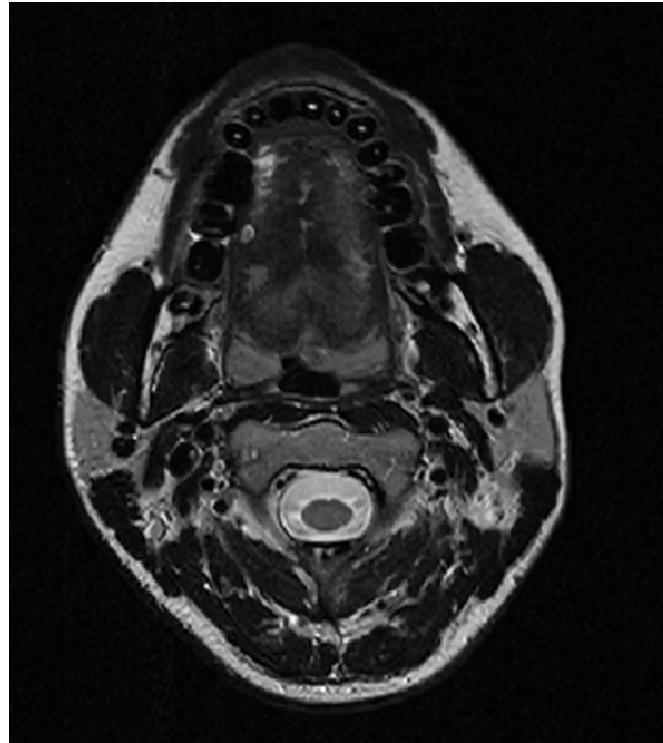
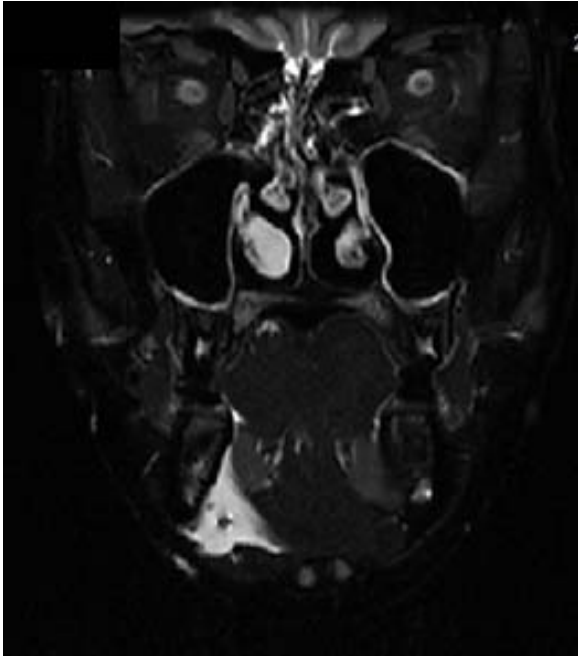
OUTCOME

- Mild pain for some days
- No dysphagia or odinophagia
- NED 1 year after the treatment



ELECTROSCLEROTHERAPY

CLINICAL CASES



1 year after the treatment
NED

Treatment of the submandibular vascular
malformation planned for next April

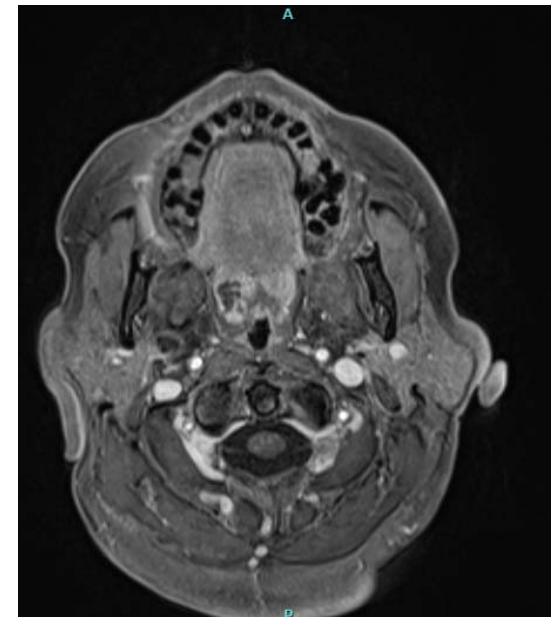
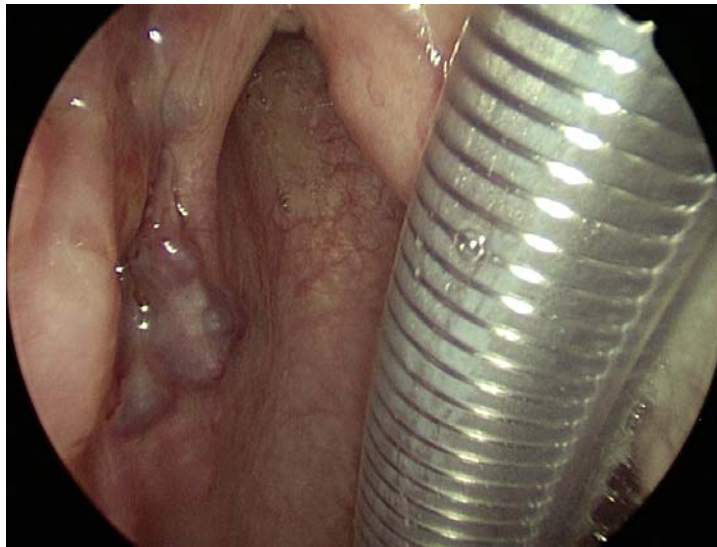
ELECTROSCLEROTHERAPY

CLINICAL CASES

Female, 56 years old

Cavernous hemangioma of the right tonsillar fossa (20 x 15 mm)

Symptomatic (pain), not bleeding lesion



Pre-operative

ELECTROSCLEROTHERAPY

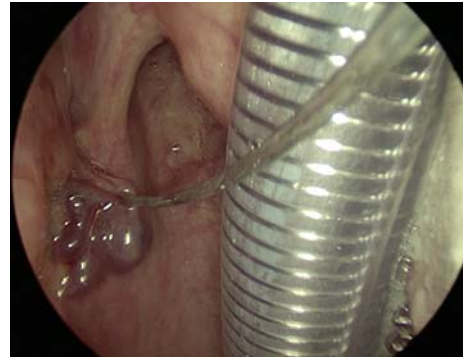
CLINICAL CASES

PROCEDURE

- Intralesional injection of 0,5 mg of bleomycin
- Electroporation of the lesion with Stinger electrode, total of 3 applications

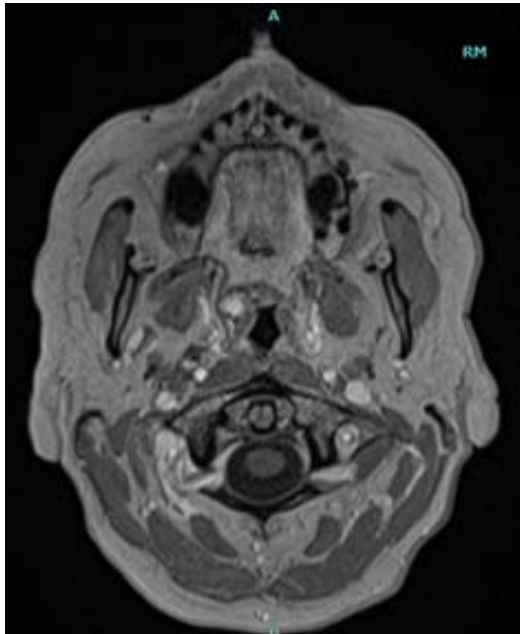
OUTCOME

- No post-operative pain
- Recurrence 1,5 months after treatment

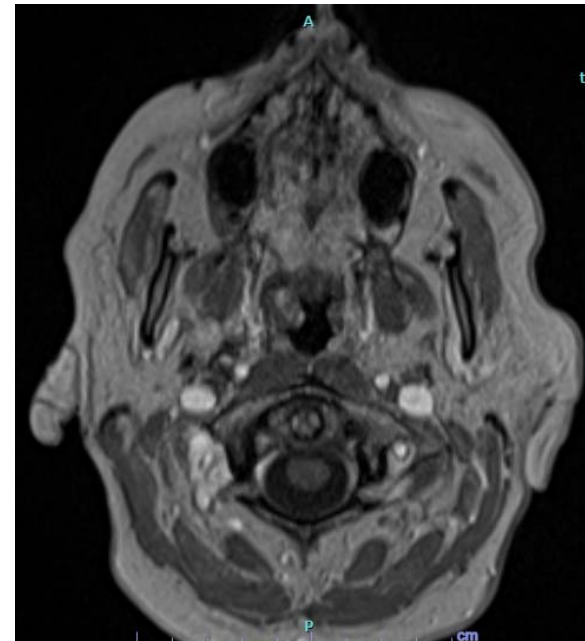


ELECTROSCLEROTHERAPY

CLINICAL CASES



After 1,5 months
(20 x 10mm)



After 8 months
(20 x 10mm)

Persistence of disease

ELECTROSCLEROTHERAPY

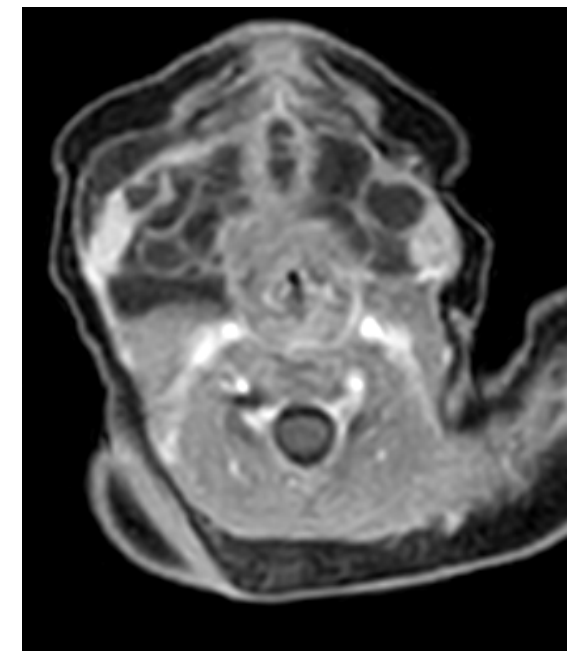
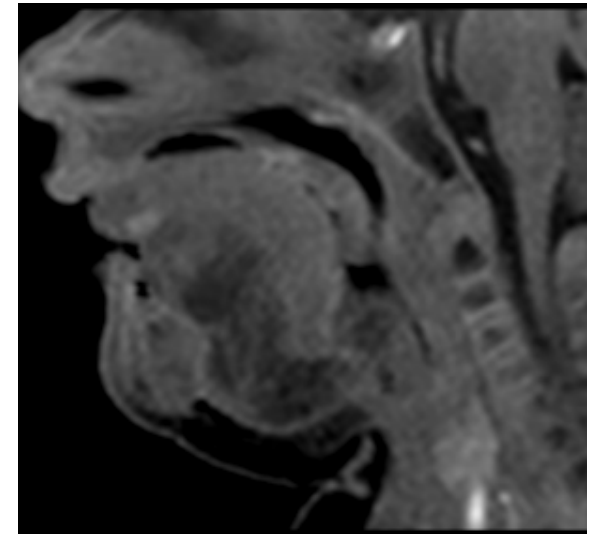
CLINICAL CASES

Female, 13 months of life

At 18 days of life appearance of non-reducible taut-elastic swelling which dislocated the tongue superiorly.

US and neck MRI → multiloculated cystic formation extending bilaterally to the sublingual space and submandibular, up to the bilateral parapharyngeal space

At 2 months → tracheostomy and sclerotherapy with Picibanil → no benefit

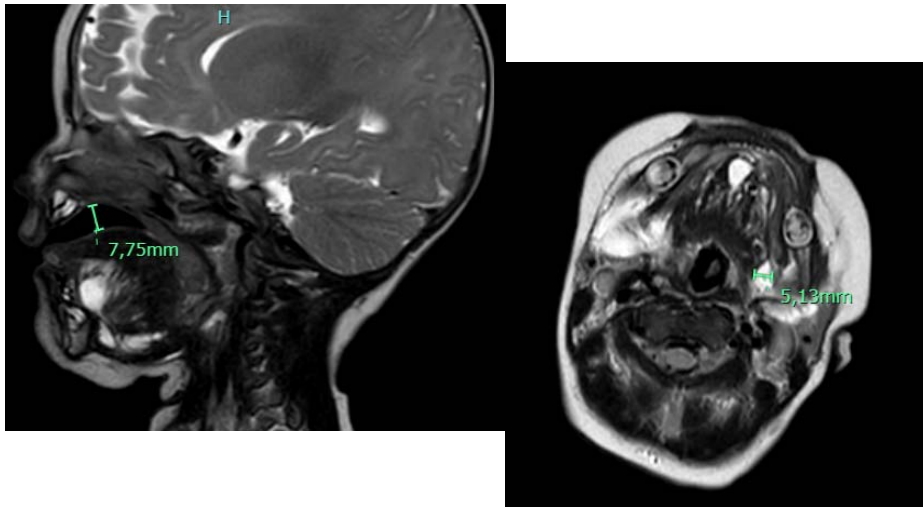


Pre-operative

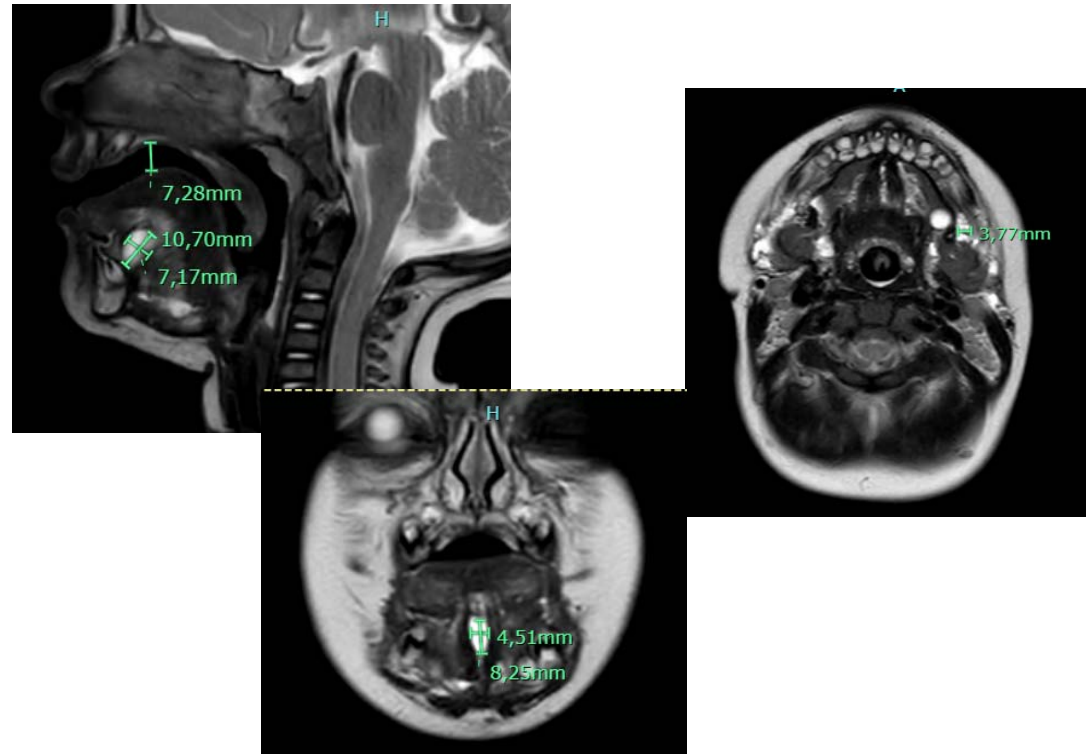
ELECTROSCLEROTHERAPY

CLINICAL CASES

At 3 months of life treatment with Sirolimus
Follow-up MRIs after 2 and 8 months show partial benefit



2 month after start of Sirolimus treatment



8 months after start of Sirolimus treatment

ELECTROSCLEROTHERAPY

CLINICAL CASES

At 13 months of life treatment of 3 lesions with Electrosclerotherapy with Bleomycin

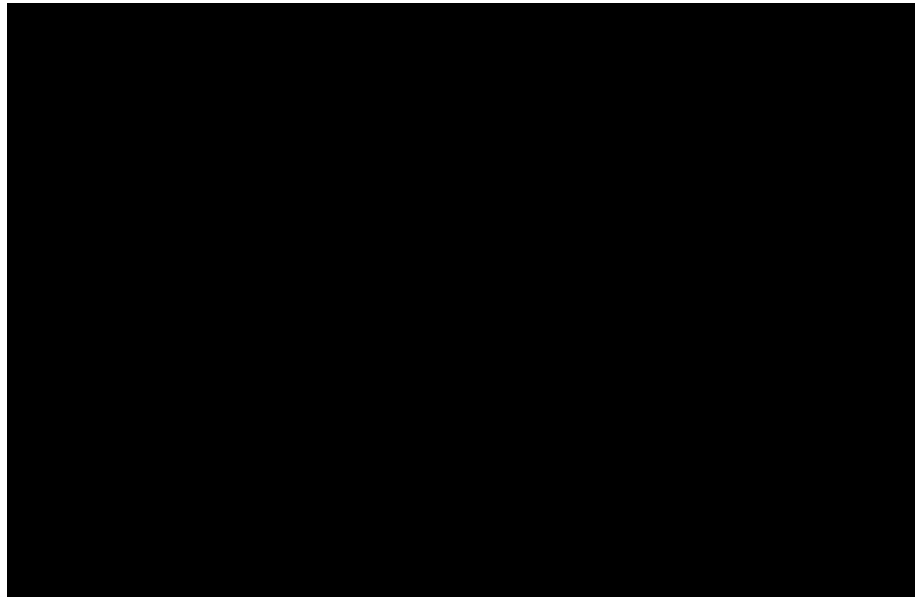
- Transcutaneous submental puncture of the left lesion under US scan, aspiration of 0.2 cc of serous fluid and injection of 0.2 cc of diluted bleomycin 0.25 mg/ml.
- Electroporation with the Finger electrode (4 applications) always under US guidance and by submental transcutaneous route.



ELECTROSCLEROTHERAPY

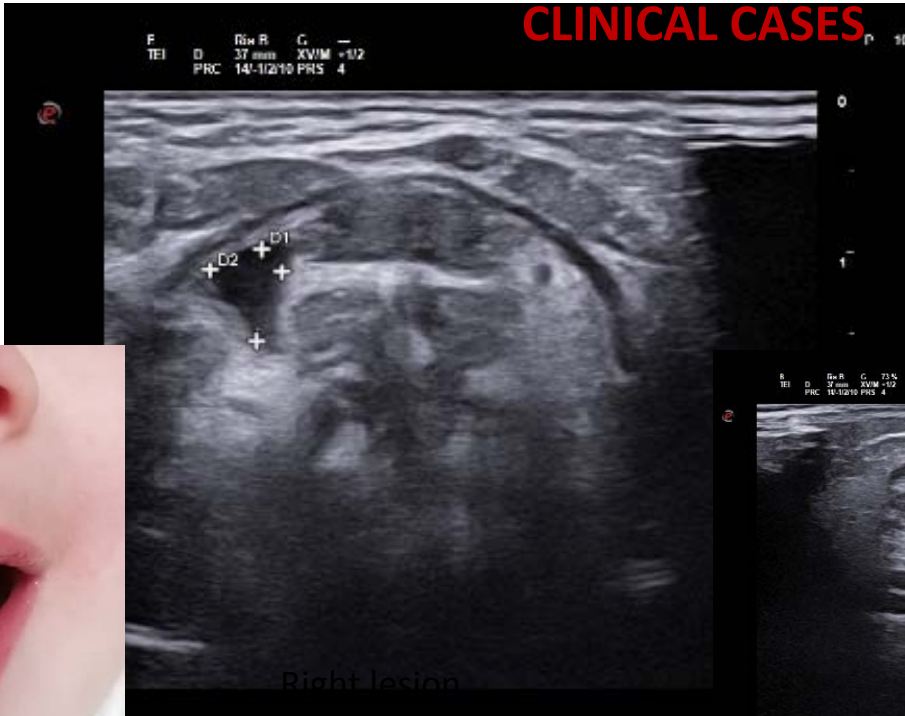
CLINICAL CASES

- Central lesion: identified and aspirated by transcutaneous submental route. Intralesional injection of 0.3 cc of diluted bleomycin.
- Electroporation performed transorally, always under US control to ensure correct positioning



ELECTROSCLEROTHERAPY

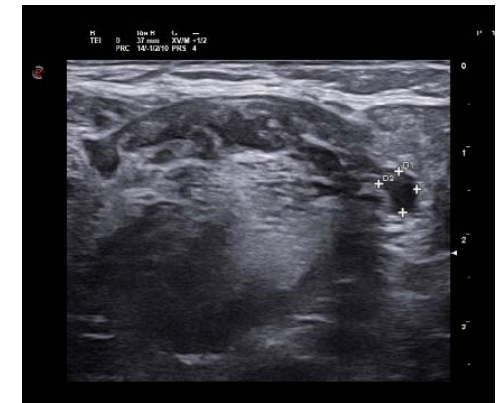
CLINICAL CASES



Right lesion

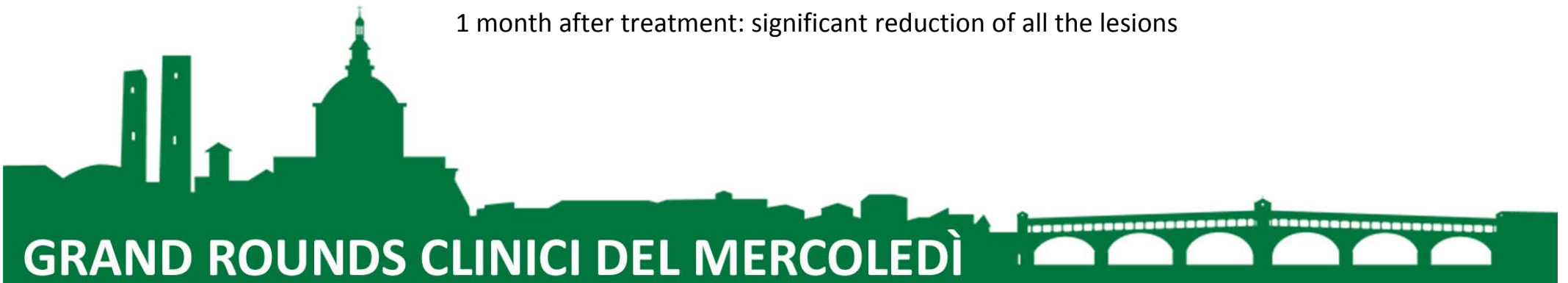


Central lesion



Left lesion

1 month after treatment: significant reduction of all the lesions



ELECTROSCLEROTHERAPY

CLINICAL CASES

Female, 19 years old

PAST MEDICAL HISTORY

Silent

PREVIOUS TREATMENT

At the age of 18 surgical removal of cystic lymphangioma of the right neck (December 2021)

CLINICAL HISTORY

Six months after surgery patient reported recurrence of the lesion.

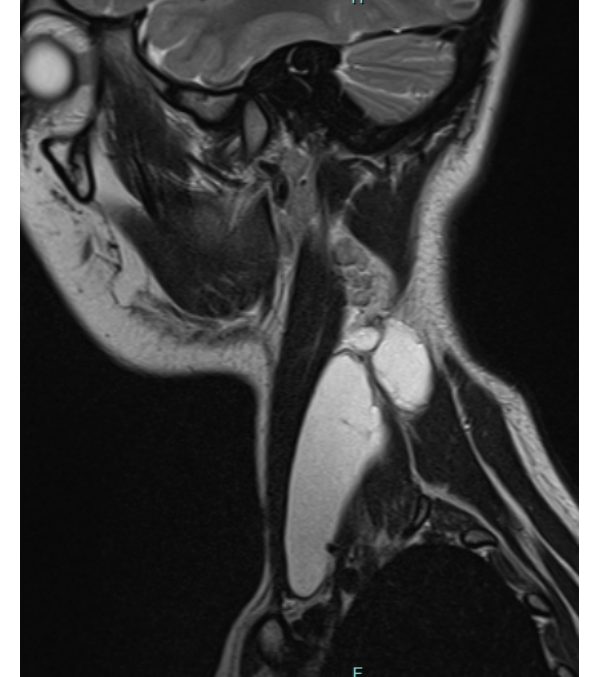
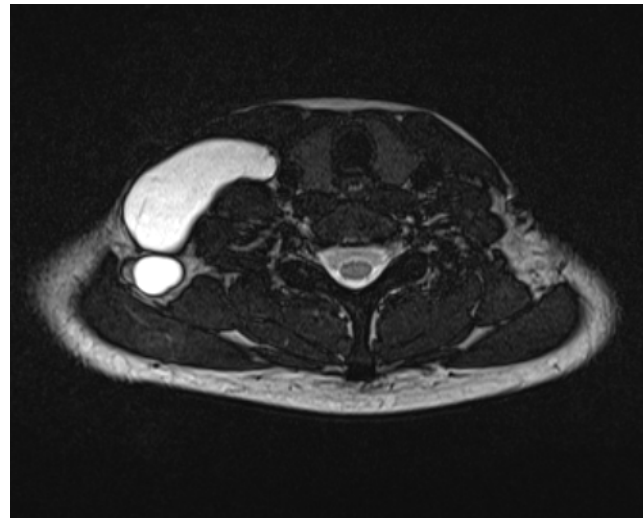
Neck MRI showed 8x 4,2 x 1,5 cm lesion in the same site of the previous lymphangioma.

One year after surgery, patient presented **two lesions** at the II and III neck level, respectively of **8x3 cm** and **6x2 cm**.



ELECTROSCLEROTHERAPY

CLINICAL CASES



MRI seven months after the surgical treatment

ELECTROSCLEROTHERAPY

CLINICAL CASES

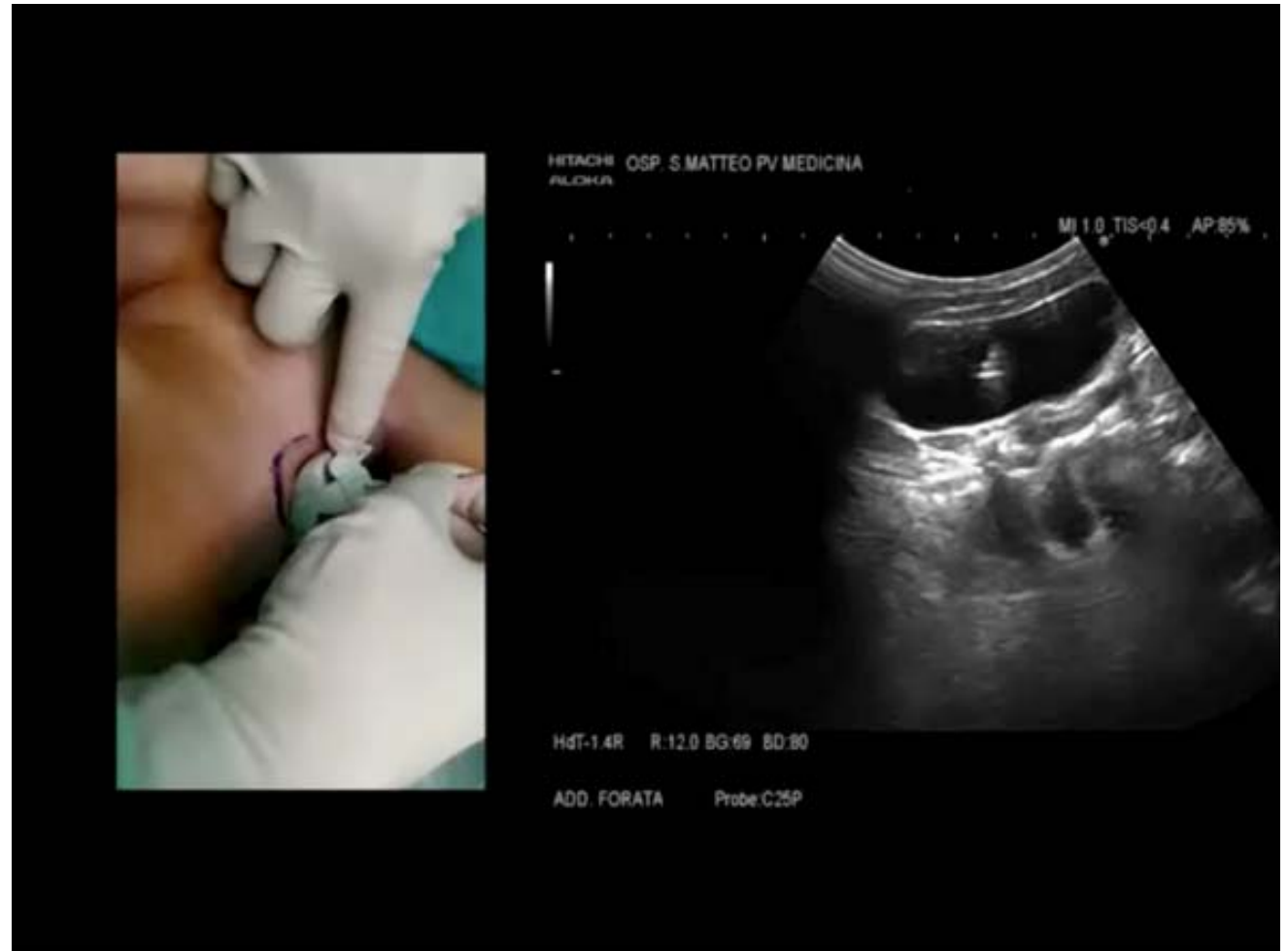


Clinical presentation one year after surgery

ELECTROSCLEROTHERAPY

CLINICAL CASES

- Transcutaneous aspiration of the liquid content of the cysts and intralesional infusion of Bleomycin for a total amount of 5 mg
- Electroporation with the N-20 HG electrode



ELECTROSCLEROTHERAPY

CLINICAL CASES



1 week after the treatment



CONCLUSIONS

- ✓ Vascular malformations in the head and neck area account for 40% of all vascular malformations cases
- ✓ Treatment is necessary in the presence of clinical symptoms, personal discomfort, cosmetic disturbance
- ✓ Sclerotherapy is currently a first-line treatment for VMs and lymphatic malformations (LMs)
- ✓ Electroporation is able to enhance the sclerosing properties of Bleomycin
- ✓ Electrosclerotherapy is technically feasible, safe with no severe side effects and no anatomical or functional damage

GRAZIE PER L'ATTENZIONE

GRAND ROUNDS CLINICI DEL MERCOLEDÌ

