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LONG TERM EFFECTIVENESS AND OUTCOMES OF VAGAL NERVE STIMULATION FOR DRUG RESISTANT EPILEPSY: A SINGLE CENTRE EXPERIENCE

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ABSTRACT

***Purpose*:** Over the past two decades, vagus nerve stimulation (VNS) has become an accepted and viable treatment modality for intractable epilepsy, both in children and adults who are not eligible for other forms of surgical treatment. Earlier studies have demonstrated short-term seizure outcomes, usually for up to 5 years; so far, few studies have reported an extended outcome.

The aim of this study is to report the seizure outcome after VNS systems implantation in a longitudinal follow-up longer than 10 years in patients with drug-resistant epilepsy followed in a single specialized epilepsy center. There were compare the outcome with respect to age of implant, aetiology, seizure type and epilepsy duration.

Methods: One hundred sixty drug-resistant epileptic patients, excluded from ablative surgery, were submitted to vagal nerve stimulation from January 2000 to January 2019. We analyzed 158 out of 160 patients with follow up more than 24 months, two patients are excluded from the study cause of fewer than three months of follow up. Median age at implantation was 14.68 years (range: 0.64 –60.87, IQR 9.07-19.93), median number of seizures pre-VNS was 100 (range: 4 – 1200, IQR: 30-200). The median age at the epilepsy onset was 1.16 years (range: 0 – 38.52, IQR: 0.41-5.58), the median years of epilepsy duration prior VNS was 11.02 years (range: 0 – 45.4, IQR: 6.76-17.03). The median time of follow-up is 60.3 months (range: 3.3 – 235,23; IQR: 36.5 – 107.36). The aetiology of the epilepsy was identified in 115/158 patients: structural in 82 pz (51,9%), infection in 12 pz (7,6%), genetic in 17 pz (10,7%), immune 4 pz (2,5 %) and unknown in 43 pz (27,2%).

The epilepsy type were identified: generalized in 33 (20.9%), combined (focal and generalized) in 39 (24.7%) and focal (focal and multifocal) in 86 patients (54.4%).

The efficacy of VNS on seizure reduction is analyzed at 3, 6, 18 months, 2, 3, 5, 10, 15 years, and at last available follow up with the previous 3 months of stimulation, in terms of responder rates and retention rate for the entire study period from stimulation onset to study completion.

‘Responders’ patients experiencing a seizure frequency reduction of 50% or more during follow-up.

Results: The seizure frequency reduction was significant in the group, as a whole between baseline and the first follow-up. The positive effect of VNS increases until 12 months (155 patients available, mean seizure rate reduction 36.38%, Wilcoxon test $p<0.001$) and the seizure reduction rate compared with baseline ($p<0.001$) persists, for each follow-up.

Univariate analysis showed a significant effect of implant age on seizure frequency reduction: the best results were observed in ‘very young’ patients (0–6 years); the largest difference is between ‘very young’ and adult patients ($p=0.03$). Lesser duration of epilepsy had positive influence on outcome: patients with longer history of seizures (>18 years) had a significantly worse clinical outcome compared with patients with less than 6 years of seizures duration ($p<0.01$).

We found no significant difference regarding the aetiology and seizure type of epilepsies in the average seizure frequency reduction. Furthermore the analysis of best responders, show that in the period since 5^{th} year of follow-up to 10^{th}, there was the higher rate of VNS end of stimulation ($p<0.01$). From evaluation of retention rate the median time of VNS stop stimulation is 83 months. Most of the patients at the end of battery service, without any remarkable change in seizures frequency, did not replacement the generator.

Conclusion: Young patients with shorter duration of epilepsy may be better candidates for VNS. The results of this study provide evidence that the VNS could induce anticonvulsant effect, and probably it is not limited to the active stimulation.

The processes that mediated these sustained changes are unknown. It is possible to speculate that the patients who reach the best response after 5 or more years of stimulation could change the neuronal network from the epileptogenic mechanism and for this, they could have stable reduction of seizure frequency, despite stimulation is in off.

For a better clinical application of the VNS, we need further scientific research to understand what processes are involved in neural networks changes.

ABSTRACT in Italiano

Scopo: Negli ultimi due decenni la stimolazione del nervo vago (VNS), è diventata una modalità di trattamento consolidata per l'epilessia farmaco resistente sia nei pazienti adulti sia nei bambini che non possono beneficiare di trattamenti chirurgici resettivi. La maggior parte degli studi condotti fino ad oggi, hanno valutato l'efficacia di questa terapia palliativa con un follow-up dai 12 mesi ai 5 anni. Solo pochi studi hanno valutato un follow-up più esteso.

Lo scopo di questo studio è quello di valutare l'evoluzione della stimolazione vagale in pazienti epilettici farmaco resistenti con un follow-up di 10 anni, studiati e impiantati in un unico centro di diagnosi e cura per l'epilessia. L'outcome è stato valutato in relazione all'età di impianto, l'eziologia, il tipo di crisi e la durata dell'epilessia.

Metodo: Dal Gennaio 2000 al Gennaio 2019, 160 pazienti con epilessia farmaco resistente, esclusi da un trattamento di chirurgia resettiva, sono stati trattati con la stimolazione vagale presso il Centro Regionale per la diagnosi e cura dell'epilessia Infantile e Adolescenziale dell'Azienda Ospedaliera Universitaria di Ancona.

Nello studio sono stati analizzati i dati di 158 pazienti su 160 totali, due pazienti sono stati esclusi in quanto presentavano al momento dello studio, un tempo di valutazione di soli 3 mesi.

L'eziologia dell'epilessia è stata identificata in 115 su 158 pazienti, suddivisa in: strutturale in 82 pz (51,9%), infettiva in 12 pz (7,6%), genetica in 17 pz (10,7%), immune in 4 pz (1,89%). In 43 pazienti (27,2%) non è stata identificata una causa della malattia. Il tipo di epilessia è stata identificata come: generalizzata in 33 pazienti (20,9%), combinata (focali e generalizzate) in 39 pazienti (24,7%) e focale (focali e multifocali) in 86 pazienti (54,4%).

L'età mediana all'impianto è di 14,68 anni (intervallo: 0,64 - 60,87, IQR 9,07-19,93), il numero mediano di crisi pre-VNS è 100 (intervallo: 4 - 1200, IQR: 30-200). La mediana dell'età dell'inizio dell'epilessia è di 1,16 anni (intervallo: 0-38,52, IQR: 0,41-5,58), la mediana della durata dell'epilessia prima della VNS è di 11,02 anni (intervallo: 0-45,4, IQR: 6,76-17,03). Il tempo di follow-up mediano è di 60,3 mesi (intervallo: 3,3-235,23; IQR: 36,5 - 107,36).

L'efficacia della VNS in termini di risposta sulla riduzione delle crisi e di retention-rate, è stata analizzata ai 3, 6, 18 mesi , 2, 3, 5, 10, 15 anni e all'ultimo follow-up disponibile. Come "responder" sono stati classificati i pazienti che presentavano una riduzione della frequenza delle crisi uguale o maggiore del 50% rispetto al baseline; quest'ultimo identificato con i 3 mesi prima dell'inizio della terapia.

Risultati: La riduzione della frequenza delle crisi dopo la stimolazione con VNS è significativa, e la risposta clinica aumenta fino ai 12 mesi post stimolazione. Su 155 pazienti disponibili alla valutazione, la riduzione media del tasso delle crisi è del 36,38% (test di Wilcoxon $p < 0,001$) e il tasso di riduzione delle crisi rispetto al basale, si è visto persistere per ogni follow-up seguente ($p < 0,001$).

L'analisi univariata ha mostrato che l'età all'impianto e la minore durata dell'epilessia prima della VNS, ha una correlazione con la risposta sulla riduzione della frequenza delle crisi. I risultati migliori sono stati osservati in pazienti che all'impianto presentavano un'età inferiore ai 6 anni, con una significativa riduzione delle crisi se paragonati ai pazienti adulti ($p = 0.03$). I pazienti che al momento dell'impianto avevano un'epilessia da più di 18 anni, mostravano una risposta ridotta rispetto a quelli con una storia di malattia minore ai 6 anni ($p < 0,01$).

Per quanto riguarda l'eziologia e il tipo di epilessia, non abbiamo riscontrato differenze significative nella riduzione media della frequenza delle crisi epilettiche dopo la stimolazione vagale. L'analisi dei pazienti "responder" mostra che nel periodo dal 5 ° al 10° anno di follow-up c'è il più alto tasso di fine stimolazione ($p < 0,01$).

Dal calcolo della retention-rate si evidenzia che il tempo mediano di stimolazione con la VNS è di 83 mesi. Dopo un periodo di almeno cinque anni di stimolazione vagale attiva, molti pazienti mostrano una riduzione stabile delle crisi. Questa risposta persiste anche dopo un periodo prolungato di sospensione della stimolazione.

Conclusione: Questo studio evidenzia che i migliori candidati alla stimolazione vagale sono i pazienti con una breve storia di epilessia e con un'età all'inizio della stimolazione inferiore ai 6 anni.

I risultati mostrano inoltre, che la VNS dopo alcuni anni di stimolazione, porta ad una riduzione stabile della frequenza delle crisi che permane anche quando questa viene disattivata. Questi dati inducono ad ipotizzare che la VNS presenta effetti anticonvulsivanti e non si limita ad una azione sulle crisi solo con stimolazione attiva.

Si può quindi ipotizzare che dopo una lunga stimolazione vagale di 5 o più anni, i pazienti che hanno risposto con una riduzione delle crisi potrebbero aver cambiato la rete neuronale, modificando stabilmente il meccanismo epilettogenico.

Per una migliore applicazione clinica della VNS, sono necessarie ulteriori ricerche scientifiche di base per comprendere quali possono essere i processi che intervengono in questi cambiamenti sulle reti neurali.

EPILEPSY: DEFINITION AND EPIDEMIOLOGY

In 2005, the League Against Epilepsy (ILAE), the main international association that deals with epilepsy both for scientific purposes and socio-cultural, released a conceptual definition of seizures and epilepsy, followed by an operational (practical) definition in 2014. Epilepsy is a disease of the brain defined by any of the following conditions: a least two unprovoked seizures more than 24 hours; one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; diagnosis of an epilepsy syndrome (1).

An "operational" definition often used for epidemiological purposes states that epilepsy is a pathological condition characterized by two or more seizures recurrent, not caused by an immediately identifiable cause (2).

The diagnosis of epilepsy is based in the first instance on a careful medical history, including details of the critical event, any warning signs, precipitating factors, type e duration of critical episodes, frequency, critical semeiology. Exams are also carried out; routine blood chemistry, neurophysiological and neuroimaging instrumental investigations that include: basal EEG, prolonged EEG after sleep deprivation and brain MRI. In selected cases metabolic tests and genetic tests can be conducted. Misdiagnosis of epilepsy has important repercussions both on the patient and on the health costs related to it.

Epilepsy is one of the most common neurological diseases. According to the WHO (World Health Organization), about 50 million people in the world are affected. More than 80 people out of 100,000 receive a new epilepsy diagnosis each year, most commonly during childhood and adolescence age (3).

In Italy, there are about 500,000 people with epilepsy (1 in 100 people) with 30,000 new cases a year, in most cases (60% of the total) with focal epilepsy (4).

Analyzing the prevalence of epilepsy in relation to different age groups, it is estimated that in Europe there are about 1 million active children and adolescents (prevalence 4.5-5 / 1,000), about 2 million adults between 20 and 64 years (prevalence 6 / 1,000) and half a million over 65 years (prevalence 7 / 1,000) (5).

About 20-30% of subjects have more than one seizure per month and 0.5-15% of patients have recurrent seizures with a frequency of less than one episode per month (6).

Thirty percent of patients continue to present seizures despite adequate anti-epileptic therapy and therefore represent the population of drug-resistant patients. There is no single definition of drug-resistance and different definitions may be appropriate depending on the form of epilepsy or the purpose for which this definition is used (7). ILAE defines *drug-resistant* as "failure of adequate trials of two tolerated and appropriately chosen AED schedules (whether as monotherapies or in combination) to achieve seizure freedom" chosen and used appropriately according to the type of epilepsy and the clinical characteristics of the patient (8).

The probability to obtaining seizure freedom is 30% after the failure of the first AED, it is reduced to 10-15% after the failure of two AEDs (in sequence or combination) and 5% after the failure of three AEDs (in sequence or combination). The use of this graduated system, in clinical research, would have offered more information about the level of drug-resistant in patients, compared to the use of the single definition established by the International League against Epilepsy.

Although many new antiepileptic drugs (AEDs) have been introduced over the last decades, offering advantages in terms of tolerability and drug interactions, most clinical trials and meta-analyses have failed to demonstrate any significant superiority in terms of efficacy of these newer AEDs as compared to older AEDs (9-11). Also, it is well established that seizure response rates to add-on AED treatment decrease with subsequent AED trials (12-14). For these reasons, overall drug response rates in patients with epilepsy have not increased significantly

over the last decades (15-17). Alternatives treatment in drug-resistant patients include experimental drugs, ketogenic diet or surgical therapy.

The different surgical approaches can be distinguished in curative and palliative therapy.

For patients with a high frequency of seizures that are not eligible for resective surgery, palliative interventions can be considered to reduce the frequency or disability seizures. Where resective surgery is not practicable, due to the impossibility of defining a single epileptogenic area or because in an eloquent cortical region, or for the presence of general contraindications to anesthesia and/or surgery or a refusal by the same patient, other therapeutic possibilities should be considered. One of these is the vagus nerve stimulation (VNS). The VNS is carried out by implanting a pacemaker-like device that exerts a central neuromodulation action through intermittent peripheral stimulation of the vagus nerve.

On the other hand, several surgical treatment options have improved epilepsy outcome for patients with drug-resistant epilepsy (DRE). Resective surgery is superior to continued drug treatment, especially in patients with temporal lobe epilepsy and an identified structural lesion (18). Additionally, established (e.g. vagal nerve stimulation, radiosurgery) and emerging (e.g. responsive neurostimulation, deep brain stimulation (DBS),stereotactic laser ablation) techniques may offer benefit for patients who are NOT candidates for resective surgery (19,17).

Since its original approval more than 20 years ago in 1997 as adjunctive therapy for reducing seizure for patient with medically refractory epilepsy, more than 100,000 patients have been implanted with VNS (20). In 1997, the US Food and Drug Administration (FDA) approved VNS as an adjuvant treatment for patients with refractory epilepsy aged ≥ 12 years, who were poor candidates or had failed to gain curative effects by resective surgery approaches (21,22).

The VNS system (Cyberonics, Inc., Houston,TX, USA) was recently approved by the FDA (2017) for use in patients over 4 years of age (23). Indeed, many clinical studies suggest that VNS therapy is effective in children as in adult patients (24).

ANATOMY OF THE VAGUS NERVE

The vagus or "migrant" nerve (from the Latin: vagus nerve), tenth cranial nerve, must be seen from the skull base up to most of the abdominal cavity. It is the longest of the twelve pairs of cranial nerves. It emerges in correspondence with the posterior lateral groove of the bulb with about ten radicles placed in vertical series in continuity with the roots of the glossopharyngeal nerve, placed above. From the emergency site, the radicles converge upwards, laterally and forwards, gathered in a single piece that obliquely crosses the arachnoid space. The nerve thus formed passes into the anteromedial part of the jugular hole, assuming a vertical course that will also remain in the neck (25). The vagus nerve then begins with its vertical direction, its extracranial path lead it through the neck and chest to the abdominal cavity. Three segments of the nerve pathway can, therefore, be described: cervical, thoracic and abdominal.

About 80% of the vagus nerve fibers are afferent. This large population of specific afferents and visceral generals conveys gustatory information from the periepiglottic portion of the pharynx and visceral information from the pharynx, larynx, trachea and thoracoabdominal viscera. In addition, a limited number of fibers carry somatosensory information from the skin of the outer ear and the neighboring region. The cell bodies of the vagal afferent fibers are found in two parasympathetic ganglia: the superior jugular ganglion and the inferior nodose ganglion, located at the jugular hole. The afferent projections of the vagus nerve are integrated at the level of the autonomic brain stem within the nucleus tractus solitarii (NTS) before projecting to other regions of the (central nervous system) CNS. We have incomplete knowledge of how VNS modulates the CNS but the brain stem plays a critical role in integrating and gating signals between the CNS and peripheral organs.

Most vagal afferents make synapses in the nucleus of the dorsal medullary complex of the vagus. The nucleus tractus solitarii receives the greatest number of vagal afferent synapses and each vagus nerve generate synapses bilaterally on the NTS. The vagal afferent synapses use common

excitatory neurotransmitters (glutamate and aspartate) and inhibitors gamma-hydroxy-butyric acid (GABA) as well as acetylcholine and a wide range of neuropeptides. The general visceral afferent fibers of the vagus carry information from the thoracic and abdominal organs, from the aortic baroreceptors and from the aortic arch chemoreceptors. These afferents play a critical role in the regulation of respiratory, digestive and cardiovascular function. These fibers lead to the sensory neurons of the nodose ganglion and retransmit them to the caudal part of the NTS. The NTS sends information to vasomotor interneurons involved in controlling blood pressure. The NTS also projects to motoneurons of the ambiguous nucleus that innervate the striated musculature responsible for the swallowing reflex, and to parasympathetic premotor neurons involved in the control of the heart rate. The caudal part of the NTS establishes connections with the periaqueductal gray matter, thus allowing the central modulation of pain. The vagal afferents, through the NTS, are also projected to the locus coeruleus (LC), the main brain noradrenergic neuromodulator system, to the raphe nucleus, the main serotoninergic neuromodulator system, to the median reticular formation and to the parabrachial nucleus. From the locus coeruleus, the information reaches the amygdala, the hippocampus and the archicortex. From the parabrachial nucleus, the afferents reach the postero-medial ventral nucleus of the thalamus that reaches the neocortex. The posteromedial ventral nucleus of the thalamus also receives special visceral afferents from the epiglottis region and projects to the gustatory cortex, to the granular layer of the insular cortex and to the inner part of the frontal operculum.

Instead, 20% of efferent vagus fibers provide parasympathetic motor fibers to all internal organs (except the adrenal glands) from the neck to the colon. These efferents originate from two pairs of nuclei of the medulla: the dorsal motor nucleus of the vagus and the ambiguous nucleus. The preganglionic fibers are then interrupted in small microscopic ganglia or at the level of agglomerations of parasympathetic ganglion cells distributed along the course of the vagus nerve, generally in the proximity of the organ that is innervated.

The vagus nerve also controls some skeletal muscles in the larynx and pharynx. The motoneurons of these efferent fibers originate in the ambiguous nucleus. Each vagus nerve also contains efferents that innervate the vocal cords, unilaterally.

Therefore the vagus nerve is important for multiple activities at the body level, including the control of blood pressure and heart rate, gastrointestinal peristalsis, vomiting, coughing, swallowing, speech and breathing. It is generally believed that the heart rate is more influenced by the right vagus nerve, responsible for the innervation of the sinoatrial node (considered the cardiac physiological pacemaker) and of the atrial chambers; the vagus nerve on the left would instead be primarily responsible for the innervations of the atrioventricular node and the cardiac ventricles. Therefore vagal anatomy promotes stimulation to the left to reduce the effects on heart rhythm.

The vagus nerve at the cervical level, detaches two branches assigned to the motor and sensory innervations of the larynx: the superior laryngeal nerve and the inferior (or recurrent) laryngeal nerve. The upper laryngeal nerve is responsible for the motor innervations of the cricothyroid muscle of the larynx and to the sensory nerve of the subglottis mucosa, the glottis, and the larynx. This branch is stimulated secondarily during the VNS and can give a sense of tension or pain in the throat. The recurrent laryngeal nerve instead gives sensitive fibers to the remaining intrinsic muscles of the larynx (with the exception of the cricothyroid) and provides cardiac, tracheal, laryngeal, pharyngeal and esophageal collateral branches, in conjunction with the superior laryngeal nerve forming the Galen loop. Given the close proximity of this nerve to the vagus, it is then influenced by the VNS causing the vibration of the left vocal cords and the hoarseness that appears during the ON phase of stimulation(26). Fig 1

HYSTORY OF VAGAL NERVE STIMULATION (VNS) AND EPILEPSY

History of Epilepsy has been present in human history, with the first record found in a Babylonian, text written 3000 years ago (27). In the late 18th century, venous hyperemia was suggested as a cause of seizures on the basis of facial flushing and bounding carotid artery pulses during seizures (28). In the 1880s, New York neurologist James Leonard Corning (1855–1923) developed instruments to decrease cerebral blood flow and electronically stimulated the human vagus nerve to abort seizures (28). However, contemporaries did not widely accept the use of his instruments due to side effects such as bradycardia, dizziness, and syncope (28). In the 1900s, animal experiments were continuously carried out to uncover the projections and functions of the vagus nerve. Bailey and Bremer (29), in 1938, reported an increased amplitude and frequency of the frontal lobe after direct stimulation of the vagus nerves of cats (30). Similarly, in 1949, MacLean et al. stimulated the vagus nerves of monkeys and found inconsistent slow waves from the frontal cortex (30). In 1951, Dell et al. reported that the stimulation of vagus nerves affected the rhinal sulcus and amygdala in awake cats (30). Based on these studies, experiments to elucidate the effects and applications of VNS were conducted. Consequently, Zabara stimulated the cervical vagus nerve of dogs for induced seizures and demonstrated an anticonvulsive effect of VNS (30). Zabara observed that repetitive electrical stimulation of the cervical vagus nerve interrupts strychnine-induced motor seizures and pentylentetrazole-induced tremors (PTZ). He induced these seizures by injecting strychnine bolus or PTZ every minute at 4-minute intervals (31). He observed that the stimulation of the vagus nerve was able to stop seizures in 0.5-5 seconds. In support of the fact that the antiepileptic mechanism was related to the central effects of vagal afferent projections, Zabara showed that the antiepileptic effects of VNS was not possible by the vagus section distal to the stimulation site. The estimated optimal stimulus parameters were: the amplitude, at about 20 V (omega resistance 1-5), the frequency of 20-30 Hz and the duration of about 0.2 ms. Finally, these data suggest that the anti-epileptic effects came

from the stimulation of demyelinating afferent fibers. From these results was possible to undertake subsequent studies for new therapeutic approach to epilepsy (32-33).

Since VNS had emerged as a promising treatment option for epilepsy, Cyberonics Inc. (Houston, TX, USA) was founded and developed a VNS device with a generator, modelled after a cardiac pacemaker in 1987(34). In 1988, the first pilot study on VNS implantation in humans for the treatment of epilepsy was carried out in four patients; two patients reported complete seizure control, one reported a 40% seizure frequency reduction, and one reported no effect (35).

Short-Term Outcomes Of Vagus Nerve Stimulation From Randomized Controlled Trials

Efficacy of VNS for the treatment of epilepsy has been examined in 4 blinded, randomized controlled trials (class I data), which are summarized in Table 1 (36-40). In a 1994 study led by Ben- Menachem and colleagues, 14 patients with partial epilepsy were randomized at multiple centers (36).

These patients received either high frequency (therapeutic) or low-frequency (sham) stimulation paradigms. At a 3-month follow-up, this study reported that high-frequency stimulation reduced seizure frequency by 25% and low frequency stimulation reduced seizure frequency by 6%. A responder to VNS therapy is commonly defined as seizure frequency reduction by at least 50%, a definition used from this point forward (41). In this study, 31% of patients receiving high frequency stimulation achieved responder status (36). In a subsequent multicenter randomized controlled trial, Handforth and colleagues (37) randomized 196 patients with partial epilepsy to receive either high-frequency stimulation or sham stimulation. Patients with high-frequency stimulation achieved 28% reduced seizure frequency whereas those with sham stimulation had a 15% decrease. Overall, 23% of those receiving therapeutic stimulation (high-frequency) achieved responder status at the 3-month postoperative follow-up (37). Amar and colleagues (38)

provided further evidence of VNS efficacy with the publication of a randomized controlled trial of VNS implantation in 17 persons, resulting in 57% of patients achieving responder status.

In the first randomized controlled trial for children with intractable epilepsy, Klinkenberg and colleagues (40) randomized patients with partial (N= 35) or generalized epilepsy (N = 6) to high output stimulation (maximum 1.75 mA) or low output stimulation (0.25 mA) for 20 weeks, followed by an add-on period of 19 weeks of high output stimulation for all patients. At the end of the randomized controlled blinded period, 16% of patients receiving high stimulation and 21% of patients receiving low stimulation achieved responder status. After the add-on phase, 26% of patients experienced at least 50% reduced seizure frequency (40). In summary, blinded randomized controlled trials for both children and adults with intractable epilepsy have demonstrated that 23% to 57% of patients typically achieve 50% seizure reduction with VNS implantation in short term follow-up (36-38,40). Additionally, these conclusions are supported by 2 non blinded randomized controlled trials (class II data -Table 1) comparing VNS stimulation parameters.

The first, a single-center study, was conducted by Scherrmann and colleagues (42) and included 28 patients, and the second, a multicenter study, was performed by DeGiorgio and colleagues (43) and included 61 patients. Scherrmann and colleagues (42) reported median seizure reduction of 30% and that 45% of patients achieved responder status. DeGiorgio and colleagues (43) reported a median seizure reduction of 26% and that 29% of patients achieved responder status.

Long-Term Seizure Outcomes For Vagus Nerve Stimulation From Retrospective And Prospective Cohort Studies

Long-term studies, including 13 prospective observational studies (class III data - Table 1), have shown progressive increases in response to VNS with increased duration of implant. (39, 41, 44)

These studies included between 16 patients and 95 patients and follow-up periods of 3 months to 64 months. As seen in Table 1, results from these studies report a median seizure reduction rate between 17% and 55% and responder rates between 21% and 54%. To further evaluate VNS response rate over time, 1 group conducted a review of VNS therapy patient outcome registry data and literature review, including 5554 patients and 2869 patients respectively (44).From registry data, 49% of patients were responders to therapy and 5.1% of patients were seizure-free at zero to 4 months post implantation. Subsequently, at 24 months to 48 months, 63% of patients were responders with 8.2% achieving seizure freedom. The authors' literary review yielded similar results with 40% of patients responders at zero to 4 months (2.6%seizure-free), and 60.1% of patients responded to therapy at last follow-up (8.0% seizure-free) (44).These studies, however, are not controlled in nature and, therefore, may be susceptible to selection bias and can overestimate long-term favorable outcomes, because patients not receiving response may be less likely to continue therapy (45).

MECHANISMS OF ACTION

The exact mechanism through which VNS exerts antiepileptic effects has not been completely elucidated yet. Although it has been demonstrated that type A fibers are the most excitable ones, followed by types B and C, respectively, it was once believed that all fibers should be stimulated to suppress seizures. Subsequently, scientists have found that C fibers are the ones responsible for the EEG desynchronization associated with epileptiform activity abolishment. Nevertheless, successive research has demonstrated that this effect was seen even after lesion of C fibers, suggesting that A and B fibers probably play a significant role (46).

Nowadays it is well established that VNS influences locus coeruleus and raphe nuclei to modulate cortical activity through alteration of noradrenergic and serotonergic projections (47).

The improve of locus coeruleus activity after electrical stimulation of the vagus nerve, demonstrated by an increase in c-fos, may provoke release of noradrenaline in the limbic circuit and activation of the dorsal raphe nucleus, which send diffuse serotonergic projections to the diencephalon and telencephalon. It is clear that VNS therapy induces variations in regional blood flow in different cortical areas including the thalamus, mesial temporal lobe, prefrontal cortex and limbic circuit, which is supported by neurofunctional imaging. Indeed, it has been postulated that modulation of some specific areas, such as the limbic circuit, could be related to better outcomes (48,49).

POTENTIAL USES AND MECHANISMS OF VNS

An exciting new application of VNS is as an anti-inflammatory treatment. Preliminary preclinical evidence suggests that VNS may attenuate the inflammatory response through activation of the cholinergic anti-inflammatory pathway (CAP) – a long loop from the vagus afferents, through the autonomic brain stem and forebrain cortical structures, and then back through the descending vagus efferents.

The CAP up-regulates HMGB1 protein, which may regulate cytokine expression, leading to anti-inflammatory effects. In recent years, Tracey et al have dedicated significant efforts to quantifying the role that VNS plays as an anti-inflammatory regulator primarily through altered regulation of acetylcholine (50-54). These findings provide strong evidence that stimulation of the vagus nerve plays a key role in peripheral cholinergic release and its putative role in suppressing inflammation. The CAP also affects the levels of acetylcholine through nicotinic acetylcholine receptors (nAChRs) (50,55).

Neurotransmitters

Many studies have been conducted with the aim of identifying which neurotransmitters are released once the potential for action in the vagus nerve fibers is generated.

From their initial studies on Woodbury and Woodbury rats they concluded that gamma-hydroxy-butyric acid (GABA), the main CNS inhibitory neurotransmitter, was involved in the mechanism of action of the VNS (56). VNS was able to reduced seizure induced by 3-mercaptopropionate and pentylentetrazole. These two compounds induce seizures by interfering with the GABAergic system.

The most significant experiment regarding the analysis of neurotransmitters implicated in the VNS was conducted by Krahl et al. who identified norepinephrine as the main neurotransmitter in the mechanism of action of the VNS (57). In the experimental rat model, the authors induced a chronic selective depletion of norepinephrine in the locus coeruleus, the main source of norepinephrine in SNC. Two weeks later they assessed the susceptibility of animals to seizures induced by maximal electroshock. It was observed that the lesion of the locus coeruleus determined the abolition of the VNS-mediated seizures suppression. The authors produced an acute inactivation of the locus by lidocaine and obtained the same results in terms of loss of anti-epileptic efficacy of the VNS.

These results are extremely important in defining the important role of noradrenaline in the mechanism of action of the Vagal Stimulator. The integrity of the noradrenergic system is important, the VNS exerts its antiepileptic efficacy, while in the absence of norepinephrine this efficacy is lacking.

The vagal afferents arrive in the nuclei of the complex dorsal area of the vagus; which represents the structure that receives the greatest number of fibers. This nucleus in turn projects to numerous bulbo-ponto-cerebellar structures, including the parabrachial nucleus, the raphe nuclei, and the locus coeruleus. The projections of the locus coeruleus widely reach the entire cerebral

cortex. A essential role for the anti-epileptic effect of the VNS is played by the connections that comprise the locus, as demonstrated by Krahl and his collaborators. The noradrenergic mechanism could also explain the antidepressant effect and improvement in the quality of life, related VNS, which were first observed in epileptic patients treated with VNS, and then specifically also in depressed patients (in fact, since 2005 the FDA has approved the VNS for the treatment of drug-resistant depression) (57).

ANTI-EPILEPTIC EFFECT OF VNS

The very first studies on animal models already showed an anti-epileptic effect of the VNS. The treatment has an anti-epileptic effect when it is able to stop the seizures. This is usually evaluated by injecting a convulsive substance into the experimental animal and then proceeding with the administration of the study treatment. The anti-epileptic efficacy concerns the symptom, but not the underlying cause. Woodbury and Woodbury had described the effect of VNS in the prevention or reduction the pentylentetrazole-induced seizures (56). Zabara had found that VNS abolishes strychnine-induced motor seizures in dogs (33). Some case reports describe the use of VNS to interrupt, in selected patients, the status epilepticus (58,59).

The anti-epileptic effect implies instead that the treatment is able to prevent the seizure, and is therefore evaluated with the ability to prevent the unexpected recurrence of seizures. Since many antiepileptic drugs are administered daily, it is assumed that the anti-epileptic effect may also be due to the achievement of constant plasma levels of the drug, and that therefore it is a chronic seizure inibition effect rather than a true anti-epileptic effect.

With the VNS new perspectives open up regarding a real, and more easily assessable, anti-epileptic effect. This is related to the intermittent stimulation mode of the vagus nerve, in relation to which it would seem that the seizure prevention also occurs during the OFF stimulation phase. Already Zabara in 1985 had observed that stimulation for one minute in the experimental animal

could produce a suppression of the seizures for the next five minutes (32). Takaya et al. studied the anti-epileptic effect of VNS by continuous and/or intermittent stimulation for 60 minutes of the vagus nerve in the experimental animal, while at intervals of 0, 3, 5, 10 minutes they injected pentylentetrazole to induce seizures (60). The results showed that the greatest anticonvulsant effect occurred after 60 minutes of continuous VNS. On the other hand, the 60-minute intermittent VNS was less effective than the continuous 60-minute VNS, but still more effective than a minor intermittent stimulation period. Takaya et al. therefore they could observe how the anticonvulsant effect declines in a time-dependent manner after VNS suspension, suggesting a cumulative effect for long-term efficacy (33).

However, it remains to be clarified whether the VNS continues to be effective once the battery has run down. The anti-epileptic effect of VNS, which could modify the neural circuits at the base of the pathology, would certainly be very probable. Most of the patients treated with VNS have a better response in the long term stimulation, where the highest percentages of responders are observed. To assess the effectiveness of the VNS, two to three years of follow-up would be necessary. This observation could rightly be interpreted as an argument in favor of the anti-epileptogenic activity of the VNS, capable of modifying pre-existing neuronal networks, and therefore assessable only in the long follow-up. However, the real anti-epileptogenic efficacy of VNS should be studied once the device has been unloaded and then turned off. Only in this way is it possible to fully explore the general anti-epileptogenic potential of neurostimulation. Seizure could increase, once the battery is exhausted, so that patients often resort to a second generator replacement intervention. Only one case report is available in the literature describing long-term seizures control after switching off the device(61). The combination of acute and chronic effects is very likely to be the basis for the effectiveness of VNS. These could include both effects on receptors, channels, currents, with acute activity; both mechanisms of gene transcription with an acute effect (transcription of c-Fos, immediate early gene product), and into chronic

(transcription of the splice variant FosB, with more delayed but more lasting activation, considered a marker of chronic neuronal activation) (62).

It is therefore likely that in the long run, this will lead to the recruitment of different networks and neuronal systems, and this could be in agreement with the fact that patients implanted with VNS show benefits even a few years after implantation and not necessarily in the short follow-up.

INTRODUCTION

The effect of VNS on seizure frequency and severity was confirmed by randomized controlled trials (37, 39, 63, 64). However, the follow-up period in three studies did not exceed 26 weeks (37, 63, 64). A meta-analysis of 74 studies with 3321 enrolled patients, proved a significant reduction of seizures at 3–12 months after surgery (36%) and an increasing effect of VNS on seizure reduction at more than one year after surgery (64).

Other studies confirmed a cumulative effect of VNS in medium-duration follow-up. For example, the median seizure reduction in 454 patients enrolled in five double-blind US studies improved from 35% to 44% at two years [65]. Similarly, a European study confirmed that treatment duration was significantly correlated with the percentage of seizure frequency reduction (42). With increased experience, studies reporting post-VNS outcomes for up to 5 years (66, 67) and studies covering follow-up periods exceeding 10–11 years have been published (68-71). The effect of VNS on seizure reduction has been discussed in combination with other aspects of VNS: post-VNS quality of life (71) and surgical problems (68).

A recent study reported a long-term follow-up, lasting from 10 to 17 years (72). The results confirmed that the benefit for patients receiving VNS implantation from systematic treatment increases over time.

The aim of this study is to report the seizure outcome after VNS systems implantation in a longitudinal follow-up longer than 10 years in patients with drug-resistant epilepsy followed in a specialized epilepsy center. The efficacy of VNS on seizure reduction is analyzed at 3, 6, 18 months, 2, 3, 5, 10, 15 years, and at last available follow up in terms of responder rates and retention rate for the entire study period from stimulation onset to study completion.

METHODS

Study population

All the patients who underwent VNS implantation for drug resistant epilepsy from January 2000 to January 2019 and followed-up at the Department of Neuropsychiatry – Epilepsy Center, AOU Ospedali Riuniti Ancona, were retrospectively identified and enrolled in this study.

Patients' data were entered into a database and were available for subsequent analysis.

The patients who underwent VNS treatment were previously ruled out as suitable candidates for resective epilepsy surgery or had already failed epilepsy surgery.

Seizure frequency was recorded in a diary by patients, parents, or caregivers and updated at each follow-up visit in our Center.

A total of 160 patients affected by drug resistant epilepsy were implanted with the VNS device between 01 January 2000 and 01 January 2019. Four patients died during follow-up, and 9 had incomplete data for medical abstraction. Overall, 146 patients were contacted and were agreed to complete the phone survey.

The data collected from the database and medical records included demographic variables, etiology of epilepsy, and results of all diagnostic workup. In particular, we collected age at seizure onset, time at VNS implantation, duration of epilepsy prior implantation, seizure type (generalized, partial, and combined) and etiology. Besides, we evaluated monthly seizure frequency data at each visit, epilepsy treatment regimen, effectiveness and side effects of VNS.

Phone surveys were conducted with patients without a recent clinic follow-up. In a structured survey, parents were asked about seizure frequency, antiepileptic drugs schedule, and side effects in the last few years. We specifically inquired about their current monthly seizure frequency, changes in seizure semiology, and current treatment regimen for epilepsy.

The seizure frequency reduction was stratified as more than 80 %, between 50 and 79 %, less than 50 %, no change, or worsening. The results were expressed based on the VNS-specific outcome scale proposed by McHugh et al.(73).Table2

We considered the group who have 50% or more seizure frequency reduction as the responder group, while others as the non-responder group.

Antiepileptic drugs (AED) regimen changes on seizure frequency over time in the setting of VNS. Many office visits were accompanied by VNS setting changes and, much more frequently, by AED regimen adjustments (medication and or dosage changes). The complexity and frequency of such changes (often multiple changes in a single visit) proved too difficult to incorporate into a meaningful analysis. We could not control all of these changes because most of the patients came from other epilepsy centers.

Stimulation parametres and setting of follow-up

During the 3-month baseline period before VNS implantation, parents were asked to keep seizure diaries. Patients were also reassessed from the clinical, neuroradiological, neuropsychological and neurophysiological perspectives, including video-recording of critical events and monitoring with ambulatory EEG. After VNS implantation, patients were monitored for 10 days in normal conditions and re-evaluated every week thereafter. The intensity of stimulation was increased in steps of 0.25 mA until the stimulation parameters reached 2 mA, at a frequency of 30 c/s, with OFF periods of 5 minutes (mins) alternating with ON periods of 30 seconds (s).

Once the target parameters of stimulation were reached the follow-up was requested of each patient every 3, 6, 12, months and every year in order to evaluate the degree of tolerance and the clinical efficacy of VNS. In a few patients, non responder, stimulation settings were switched to a fast stimulation pattern (ON period 30 s, OFF period 3 mins to 1.8 mins) after one year of

follow-up. In our experience, we seldom found remarcable different results by changing stimulation parameters.

Clinical efficacy was determined by comparing the seizure frequency during the past 3 months of follow-up with the seizure frequency during the pre-implantation period, using the following formula: (seizures per month on VNS – baseline seizures per month) /(baseline seizures per month) × 100.

Surgical procedure

Most patients underwent a standard VNS procedure. For this, the patient is placed in a supine position with a shoulder roll beneath the scapulae to provide mild neck extension. With the head partially rotated to the right, a 6-cm curvilinear chest incision is made in the superior lateral region of the chest following the natural anatomical profile of the armpit. The underlying fat and pectoralis fascia are dissected to create a subcutaneous pocket for the pulse generator. Subsequently, a 6-cm transverse neck incision is made 2 cm above the clavicle, and the deep cervical fascia is opened to identify the neurovascular bundle afters separating the platysma. The vagus nerve is generally found deeper and medial to the internal jugular vein and lateral to the common carotid artery. Approximately 4 cm of the nerve trunk is dissected and superficialized. A tunnelling tool is used to create a subcutaneous tract between the two incisions to construct a passage for the lead connector pins. Positioning the three-electrode coils around the nerve is more easily performed, with two surgeons working simultaneously and using magnifying lenses. Once the cables are connected to the generator, the lead test is carried out to evaluate the impedance and the output current. An intradermic suture of both wounds concludes the operation after a standard multilayer closure. The procedure usually lasts approximately 1 hour and is typically performed under general anesthesia.

The device is tested for function and the electrode impedance is checked prior to leaving the operating suite. Patients receive antibiotic therapy before and 3 days after surgery. From 2002 to

2012, the surgical technique has been modified by using a single cervical incision as described by Glazier et al. (74). The neck incision is made 1–2 cm above the clavicle. The upper retraction of the wound allows exposure of the vagal nerve while the lower retraction is used to create a subclavicular pocket to host the generator at a distance of approximately 7 cm from the electrodes. The pulse generator is placed underneath the pectoralis major muscle and secured to the fascia of the intercostal musculature. The electrode leads are placed around the vagus nerve, as described above. Patients are generally discharged 48–72 h after surgery (75).

Side effects

The side effects of the short and long term VNS are usually of the mild entity.

The surgical procedure was well tolerated in all cases without noticeable complications during and after surgery. In 11 patients significant pain was reported at the site of implantation of the generator. The aesthetic damage arising from the size of the stimulator was acceptable in all cases. Adjustment of the stimulation parameters to the final selected patterns could always be started after seven days and pursued without significant problems weekly thereafter. Only one patient experienced transient pharyngodynia with a sensation of nausea when the stimulation intensity reached 0.75 mA. A change in the vocal timbre when the stimulation is in ON was reported in 75% (120/160) of patients during the stimulation period. However, this was not a significant problem in anyone.

In 1 out of 3 patients who were switched to a fast stimulation cycle, increased salivation and intense asthenia developed. These symptoms rapidly receded after the restoration of standard stimulation parameters.

Common adverse effects such as cough, dyspnea, hoarseness, voice alteration are usually transitory and due to the stimulation of the inferior (recurrent) laryngeal nerve and directly related to the frequency of stimulation (75). These side effects tend to improve or disappear with

time or reducing the stimulation frequency from 30 Hz to 20 Hz. Fifty-two out of 160 patients reported hoarseness and coughing during the setting phase when increasing the stimulation parameters. Both these events resolved in 1 to 2 days after the adjustment of the stimulations.

Lead breakage must be considered a significant complication, in our series, occurred in 9% of patients (15/160) three years or more after surgical implantation without a history of trauma or drop-attacks. The breakage, has been discovered accidentally performing a lead test that showed high impedances and did not present as an absence of perceived stimulation.

For 13 out of 15 patients with lead breakage the clinical results were satisfying, it was mandatory to perform a new surgical procedure after removing all the previous device. The surgical procedure was well tolerated in all cases without complications.

One patient was explanted for jaw pain in active stimulation at 18 months of VNS activation and one patient responder (98% of seizure reduction) at 10 years of follow-up VNS was inactivated for sleep apnoea.

Statistical Analysis

Statistical analysis was conducted using STATA/IC version 15.

Categorical variables were compared using two-tailed Pearson's chi-square test or Fisher exact test, while differences in continuous variables were evaluated by Kruskal-Wallis test with a post hoc comparison with Mann-Whitney's U test using a Bonferroni correction for multiple comparison.

The Kaplan-Meier survival curves were made to assess the time to VNS failure and turned off stimulation.

The impact of other epilepsy related factors including age at implantation, epilepsy and seizure type, duration of epilepsy prior implantation were evaluated using Pearson's chi-square test or Fisher exact test or Wilcoxon signed-rank test. A p-value ≤ 0.01 was considered statistically significant.

RESULTS

A total of 160 patients with epilepsy were identified as having VNS implantation between 2000 and 2019. Four patients were confirmed to be deceased. Nine patients were lost to follow-up before study year 5 (at last follow-up one was seizure free at three years, two were 50% of seizure reduction at two years and six were non responder after 2 years, 18 months and 6 months). The probable reason that patients with good outcomes dropped out was their choice to continue follow-up care in their native country or closer to their home (our center was the first in the country to start systematic VNS implantation). Three patients died before study year 15th (one responder at 10 years of follow-up, was 92% of seizure reduction and two were non responder). One patient died at 6 years of follow-up and was responder, 75% of seizure reduction at last visit. In two patients, the cause of death was unrelated to epilepsy, one died for drowning and one for pulmonary infection. The possibility of SUDEP (Sudden Unexpected Death in Epilepsy) could not be excluded in two patients who reportedly died from heart failure.

Of the remaining 147 patients, were contacted and were agreed to complete the phone survey. In a structured survey, parents were asked about seizure frequency, antiepileptic drugs and side effects in the last few years. In Fig 3 were reported number of patients and outcome per follow-up.

Two patients are excluded cause of fewer than 3 months of follow up.

We analyzed 158 patients, (85 males,73 females) actual median age is 26 years (range: 9 – 66, interquartile range – IQR 21-33), median age at implantation was 14.68 years (range: 0.64 – 60.87, IQR 9.07 – 19.93), median number of seizures pre-VNS was100 (range: 4 – 1200, IQR: 30 – 200). The median age at the epilepsy onset was 1.16 years (range: 0 – 38.52, IQR: 0.41 – 5.58), the median years of epilepsy duration prior VNS was 11.02 years (range: 0 – 45.4, IQR: 6.76 – 17.03). The median time of follow-up is 60.3 months (range: 3.3 – 235,23; IQR: 36.5 – 107.36). The patients had received a median of three AEDs (range:1 – 6) before implantation.

Aetiology and Epilepsy type

The aetiology of the epilepsy was identified in 115/158 patients. Structural aetiology was in 82 pz (51,9%): prematurity and perinatal anoxic/ischemic lesions in 26 (16.5 %), vascular malformation 3 (1.89 %), cortical dysplasia 36 (22.78%) post traumatic 2 (1.26%), tuberosclerosis in 15 (9.5 %); infection aetiology was in 12 pz (7,6%): encephalitis/meningitis in 7 (3,8 %), herpes virus infection 6 (3,79%), Toxoplasma gondii infection 1 (0,63%); genetic aetiology was in 17 pz (10,7%): Dravet syndrome in 12 (7,59 %), Rett Syndrome /CDKL5 2 (1.26%), chromosomal abnormalities 4 (2,5%) immune etiology in 4 pz (2,5 %): febrile infection-related epilepsy syndrome (FIRES) 3 (1.26 %), probably autoimmune 1(0.63 %).The aetiology was unknown in the 43 of the patients (27,2%).

All patients underwent routine and prolonged video-EEG evaluation. The diagnosis of epilepsy type is made supported by the finding of typical ictal and interictal EEG discharges and on clinical grounds.

The epilepsy type were identified in all patients: generalized in 33 (20.9%), combined (focal and generalized) in 39 (24.7%) and focal (focal and multifocal) in 86 patients (54.4%).

Regarding the aetiology and seizure type of epilepsies we found no significant difference in the average seizure frequency reduction.

Duration of epilepsy

Since the first seizure to VNS implant is the duration of epilepsy. The median years of epilepsy duration before VNS were 11.02 years (range: 0 – 45.4, IQR: 6.76 – 17.03). Duration of epilepsy is a continuous variable, strictly linked to the age of implant and then difficult to analyse. In order to assess its effects on clinical outcome, independently from age, we divided the patients

into four classes of duration: less than 6 years, from 6 to less than 12 years, from 12 to less than 18 years and more than 18 years.

Average seizure frequency reductions after 3 and 36 months of VNS were 27% and 45% (<6 years), 25% and 40% (6 – 12 years), 24% and 39% (12 – 18 years), 17% and 28% (>18 years). Patients with a longer history of seizures (>18 years) had a significantly worse clinical outcome compared with patients with less than 6 years of seizures duration ($p<0.01$).

Patients with a duration between 6 – 12 and 12 – 18 resulted in an intermediate cycle ($p=0.03$).

Age at implant

The average age at implant was 14.5±11 years (IQR9.1 – 19.9 years). According to Alexopoulos et al. Three classes of age were considered (76):

1. Pre-adolescent 0–12 years, 62 patients (39%)
2. Adolescent 13–18 years, 42 patients (27%)
3. Adult: more than 18 years, 54 patients (34%)

According to with Zamponi et al. and Blount et al., we made a further subdivision of pre-adolescent group in 'very young' (0 – 6 years, 21 patients, 13%) and children (7–12 years, 41 patients, 26%;) (77-78).

Pre-adolescent (0 – 12 years) had a better clinical outcome than adult patients (The chi-square statistic is 10.5015. The p-value is .001193) and slightly better than adolescents (13–18 years; The chi-square statistic is 6.9534. The p-value is .008366.).The best results were observed in 'very young' patients (0 – 6 years); the largest difference is between 'very young' and adult patients ($p=0.03$); 'Very young' patients (0 – 6 years) having the highest average seizure frequency at baseline (293 seizure/month) show the best percentage of seizure reduction, 34% at 3 months (125 seizure/month).

Clinical outcome

Description of patients outcome is described at the time of 3, 6, 12, 18, 24, 36 months and 5, 10, 15 years of follow-up. The responder rate (patients experiencing a seizure frequency reduction of 50% or more) was 28,5%, 35,5%, 42,5%, 44,5%, 46,5%, 48,5%, 50%, 74,5%, 82% respectively in each follow-up .Fig 4: responders rate

In the last follow-up the responders' rate is very high due to the reduction of patient number; most of non responders patient did not reimplant the generator when the battery was in the end of service.

After 3 months of VNS, the mean seizure rate reduction was 24.7% (Wilcoxon test $p<0.001$). The positive effect of VNS increases until 12 months (155 patients available, mean seizure rate reduction 36.38%, Wilcoxon test $p<0.001$) and the seizure reduction rate compared with baseline ($p<0.001$) persists, for each follow-up.

After 3 months, two patients were seizure-free (1.2%); the rate of seizure-free patients tends to increase over time 3.1% at 12 months, and 3.7 % at 5 years.

Retention rate

Patients who continues to have the VNS stimulation were 99.35%, 96.76%, 94.74%, 85.68%, 66.86%, 38.37%, and 26.55% respectively at 12, 18, 24, 36, 60, 120, and 180 months (see Tab 3).

The median time of VNS turned OFF is 83 months. No statistical significant differences in time to VNS turned OFF were observed with regard to epilepsy type, seizure type, age at implantation, and duration of epilepsy prior implantation. Fig 5

DISCUSSION – CONCLUSION

Long-term intermittent VNS is an increasingly accepted method for treating drug-resistant partial epilepsy in patients who are not eligible for other forms of surgical treatment. The improvement in seizure control appears to be similar to that yielded by the use of newer drugs, without significant systemic and cognitive side effects (39,79,80).

Our study confirms the efficacy of VNS in seizure reduction in drug-resistant epileptic patients. Results, in seizure reduction and percentage of the responder, are similar to those of the main published series (63, 81). Average seizure reduction 31,9%, Wilcoxon test $p<0.00001$. Also, the percentage of seizure-free patients is in line with these add-on trials of new AEDs in children with drug-resistant epilepsy.

The significant finding of this study is that young age at implant is strictly linked with good clinical outcome. In other studies, better efficacy was observed when VNS therapy was started earlier (82; 83, 84). These findings could become important in selecting the right candidates for VNS therapy.

Very young age resulted in important predictors of favorable clinical outcome. Children had clinical results significantly better than adults. Our data show that pre-adolescent (0 – 12 years) had a better clinical outcome than adult patients (The chi-square statistic is 10.5015. The p-value is .001193) and slightly better than adolescents (13–18 years; The chi-square statistic is 6.9534. The p-value is .008366). The best results were observed in 'very young' patients (0 – 6 years); the most significant difference is between 'very young' and adult patients ($p=0.03$). This finding, already hypothesized by other authors (85), in our series is supported by a direct assessment amongst the different groups of age studied with the same protocol and is confirmed by statistical analysis (univariate and survival analysis). In terms of percentage reduction of seizure amongst the four classes of age, the best results are obtained in patients less than 6 years old (84-85).

The short duration of epilepsy before the implant is another factor strongly associated with good prognosis. This result supports the conclusion of previous works that suggest the need for an earlier, rather than later, initiation of VNS therapy in patients with drugs-resistant epilepsy (85). Helmers et al. evidenced the relationship between short duration of epilepsy and clinical efficacy of VNS, retrospectively comparing early treatment group (less than 6 years of epilepsy) versus late treatment group (more than 6 years). Patients treated earlier with VNS were twice as likely to report no seizures as patients who had seizures for more than 6 years, before they received VNS therapy (83). Analogous conclusions were reported by Renfroe and Wheless who compared patients with short (<5 years) and long (>5 years) duration of epilepsy (82). The importance of young age and duration of epilepsy was already evidenced by You et al. who reported the long-term outcome of VNS in 28 children with refractory epilepsy. They found that seizure reduction rate, tended to be inversely related to the seizure duration, before VNS implantation and to the age at the time of VNS implant (86).

From recent meta-analysis Wang et al. provide evidence for the preoperative evaluation of VNS, those drug-resistant epilepsy patients with shorter duration of epilepsy may be better candidates for VNS and they are easier to help attain seizure reduction, rather than those who are younger at onset and implantation (87).

However, it should be noted that in those series, as in our, short duration of epilepsy is strictly linked with young age. In order to assess the effect of duration of epilepsy on clinical outcome without the influence of age, further studies should analyse two groups of patients with the same age (possibly adult) and different duration of epilepsy.

Regarding the aetiology and seizure type of epilepsies, we found no significant difference in the average seizure frequency reduction.

Most of the patients have the battery of vagal nerve stimulation on end of service, between fourth and seventh year. The off battery time is usually given by vagal nerve stimulation model, the stimulation features, the system electrical resistance and the use of the magnet.

The older and most recently model systems do not have a real alarm for the battery low.

The analysis of best responders show that 8 out of 64 responders (12.5%), stop the VNS stimulation during the period 36 mos – 5 years FU, 17/49 (35%) in 5 – 10 years FU and 6/32 (19%) in 10 – 15 years FU. In the period since 5th year of follow-up to 10th there was a higher rate of VNS end of stimulation ($p<0.01$). From evaluation of retention rate the median time of VNS stopped stimulation is 83 months. The responder patients who realized the battery end of service in routine follow up, without any remarkable change in seizures frequency, usually decided to take a waiting period, before planning surgical operation to replace the generator, in agreement with the medical staff. We observed that the majority of the patient remains off instead to replace the battery, because they did not increase the frequency of the seizures. Three patients only replaced the generator of vagal nerve stimulation after 3-7 month of a waiting period for seizures increase.

The results of our study provide evidence that the VNS could induce anticonvulsant effect and probably is not limited to the active stimulation. The processes that mediate these sustained changes are unknown, but the persistence of the anticonvulsant effects suggests that VNS induces long-term changes in neural activity. A study by Naritoku et al. demonstrated VNS-induced expression of fos protein immunoreactivity in several regions of the brain that are important in epileptogenesis, including limbic structures, thalamic nuclei, and brainstem noradrenergic nuclei (88). Fos is a protein that signals transcription of other genes and may mediate long-term activity-induced changes in neuronal behaviour (89).

In summary, electrical VNS induces persistent neural effects that increase the threshold for seizure initiation. They suppose that VNS may be potentially used as preventive, as well as abortive therapy for seizures. These findings suggest that it is possible to increase the efficacy of intermittent VNS by increasing the duration of the stimulation (60).

Fraschini and Marrosu evaluated the phase lag index (PLI) used to estimate functional connectivity between EEG channels and the minimum spanning tree (MST), was computed in

order to characterize VNS-induced alterations in network topology, in a bias-free way. From this study they confirmed the hypothesis that VNS induces a network re-organization in the brains of epilepsy patients that respond to stimulation, and therefore suggested that functional network modifications could be used as a marker in monitoring the efficacy of VNS treatment, in terms of anticonvulsant and potentially antipsychotic and mood-stabilizing properties (90).

In the recent experiment with adult male Sprague-Dawley rats, the author shows that VNS: enhances novelty preference, alters the hippocampal cortical and blood epigenetic transcriptomes, modulates neuronal plasticity and stress-response signaling genes epigenetically (91). From our analysis, we speculate that the patients who reach the best responders after 5 or more years of stimulation could change the neuronal network from the epileptogenic mechanism and for this, they could have stable reduction seizure frequency despite stimulation is in off.

TABLES –FIGURES

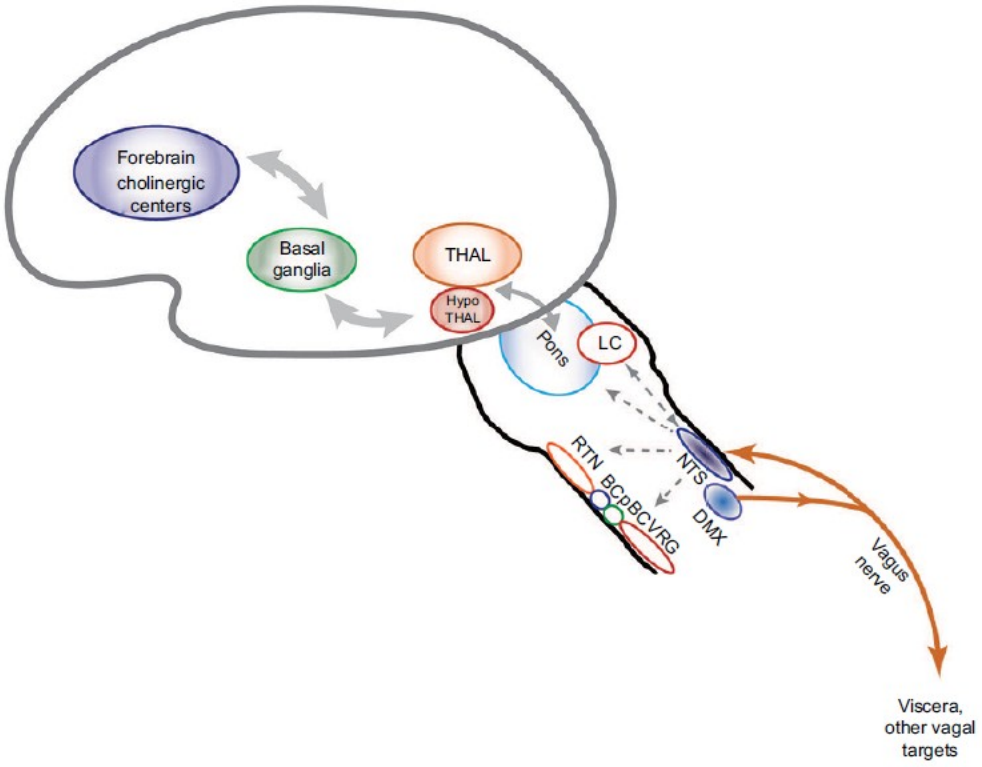

FIG 1 Notes: Stimulation of the vagus activates ascending pathways that alter neural circuits in the brain stem, midbrain, and cortex. Regions that are impacted by vagus nerve stimulation based on past research are included in this diagram.
Abbreviations: NTS, nucleus tractus solitarii; DMX, dorsal motor nucleus of the vagus; LC, locus coeruleus; THAL, thalamus; HypoTHAL, hypothalamus; RTN, retrotrapezoid nucleus; BC, Bötzinger complex; pBC, preBötzinger complex; VRG, ventral respiratory group.

Table 1
Class I, class II, and class III evidence of vagus nerve stimulation efficacy in epilepsy treatment

Class I Evidence: Blinded, Randomized Controlled Trials

Study	N	Seizure Type	Comparison	Follow-up	Number of Centers	Mean Seizure Reduction, %	Patients With Greater Than 50% Reduction, %[a]
Ben-Menachem et al,[19] 1994	114	Partial	High vs low stim.	3 mo	Multi	25 (high) vs 6 (low)	31
Handforth et al,[20] 1998	196	Partial	High vs low stim.	3 mo	Multi	28 (high) vs 15 (low)	23
Amar et al,[21] 1998	17	Partial	High vs low stim.	3 mo	Single	71 (high) vs 6 (low)	57
Klinkenberg et al,[23] 2012	41	Mixed	High vs low stim.	3 mo	Single	16 (high) vs 21 (low)	26[b]

Class II Evidence: Nonblinded, Randomized Controlled Trials

Study	N	Seizure Type	Comparison	Follow-up	Number of Centers	Median Seizure Reduction, %	Patients with Greater Than 50% Reduction, %
Scherrmann et al,[25] 2001	28	Mixed	2 Stim. paradigms	NR	Single	30 (overall)	45
DeGiorgio et al,[26] 2005	61	Partial	3 Stim. paradigms	3 mo	Multi	26 (overall)	29

Class III Evidence: Prospective Observational Studies (>10 Patients)

Study	N	Seizure Type	Notes	Follow-up	Number of Centers	Mean or Median Seizure Reduction, %	Patients with Greater Than 50% Reduction, %
Ben-Manachem et al,[56] 1999	64	Mixed		3–64 mo	Single	NR	45
Parker et al,[57] 1999	15	Mixed	Children with encephalopathy	1 y	Single	17	27
Labar et al,[58] 1999	24	Gen		3 mo	Single	46	46
DeGiorgio et al,[44] 2000	195	Mixed		12 mo	Multi	45	35
Chavel et al,[59] 2003	29	Partial		1–2 y	Single	53	54 (at 1 y)
Vonck et al,[60] 1999; Vonck et al,[61] 2004	118	Mixed		>6 mo	Multi	55	50
Majoie et al,[62] 2001; Majoie et al,[63] 2005	19	Mixed	Children with encephalopathy	2 y	Single	20.6	21
Huf et al,[64] 2005	40	NR	Low IQ adults	2 y	Single	26	28

Class III Evidence: Prospective Observational Studies (>10 Patients)

Study	N	Seizure Type	Notes	Follow-up	Number of Centers	Mean or Median Seizure Reduction, %	Patients with Greater Than 50% Reduction, %
Kang et al,[65] 2006	16	Mixed	children	>1 y	Multi	50	50
Ardesch et al,[66] 2007	19	Partial		>2 y	Single	25 (at 2 y)	33 (at 2 y)
Ryvlin et al,[67] 2014	112	Partial	VNS + BMP vs BMP	2 y	Multi	23 (at 1 y)	32 (at 1 y)
Fisher et al,[42] 2016	20	Mixed	AutoStim trial	1 y	Multi	47.3	50
Boon et al,[43] 2015	31	Mixed	AutoStim trial	1 y	Multi	NR	29.6

Abbreviations: BMP, best medical practice; Gen, generalized; Multi, multiple; NR, not reported; Stim, Stimulation.
[a] Refers to "high" stimulation group only.
[b] Refers to add-on period results with all participants switched to high-stimulation.
Adapted from Englot DJ, Chang EF, Auguste KI. Vagus nerve stimulation for epilepsy: a meta-analysis of efficacy and predictors of response. J Neurosurg 2011;115(6):1250; with permission.

Class I IA IB	80–100% reduction in seizure frequency Improvedictal or posticta activity No improvement ictal or posticta activity	Class I 80–100% reduction in seizure frequency
Class II IA IB	50–79% reduction in seizure frequency Improved ictal or posticta activity No improvement ictal or posticta activity	Class II 50–79% reduction in seizure frequency
Class III IIIA IIIB	<50% reduction in seizure frequency Improved ictal or posticta activity No improvement ictal or posticta activity	Class III <50% reduction in seizure frequency
Class IV	Magnet benefit only	
Class V	No improvement	Class V No improvement

Table 2 McHugh's classification and Mchugh'class. modified for all seizure type after VNS

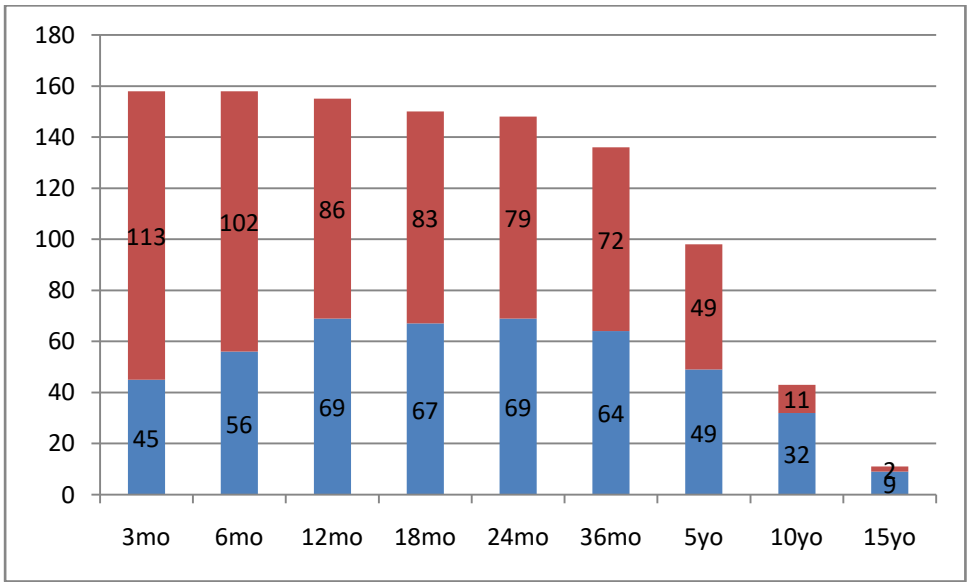

Fig 3 were reported number of patients and outcome per follow-up
Responder blu (reduction seizure frequency>=50%)
non responder red (reduction seizure frequency<50%)

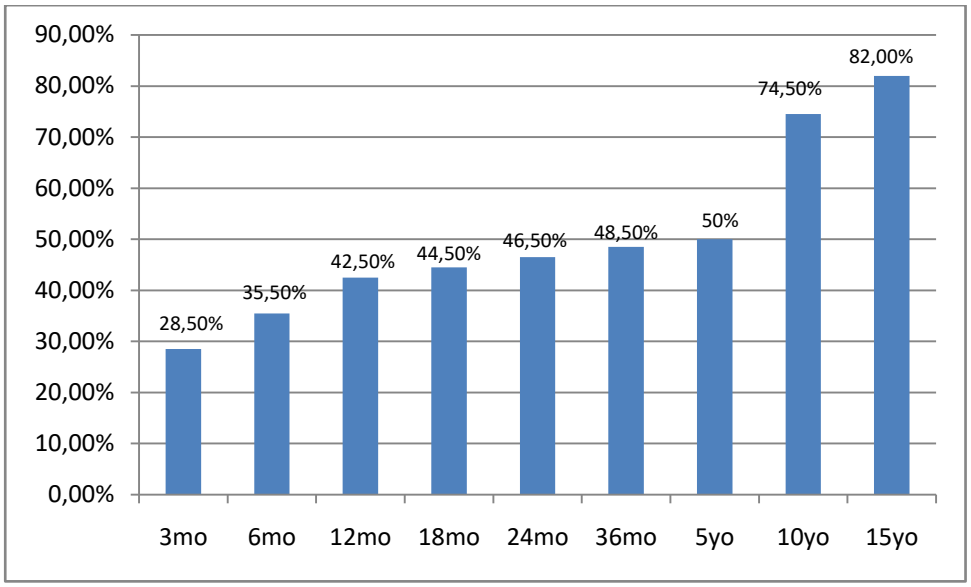

Fig 4 : Response rate

Time (months)	% Survival Function	95% CI (Confidence Interval)
12	99.35%	95.51 - 99.91%
18	96.76%	92.39 -98.64%
24	94.74%	89.75 – 97.34
36	85.68%	78.87 – 90.42
60	66.86%	58.14 – 74.16
120	38.37%	29.29 – 47.37
180	26.55%	17.96 – 35.92

Tab3: retention rate. Patients who continues to have the VNS stimulation

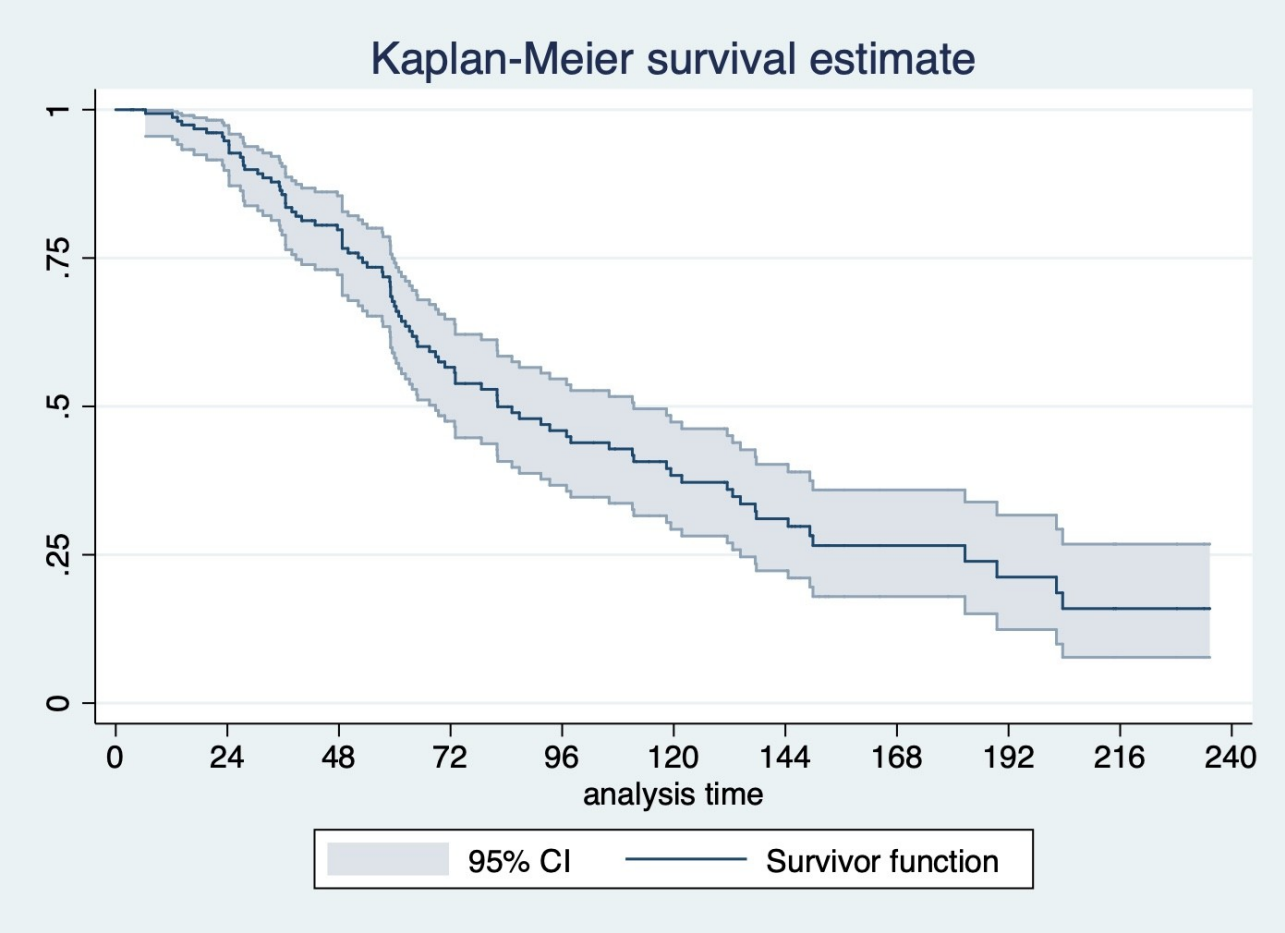

Fig5

Fig 5 Patients who continues to have the VNS stimulation

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