# Clinical and instrumental assessment of the corneal healing in moderate and severe neurotrophic keratopathy treated with rh-NGF (Cenegermin)

**Anna M Roszkowska[1,2], Leandro Inferrera[1], Emanuela Aragona[3], Romana Gargano[4], Elisa I Postorino[1] and Pasquale Aragona[1]**

## Abstract

**Purpose:** To evaluate corneal healing in patients with moderate and severe neurotrophic keratitis (NK) treated with topical rh-NFG (Cenegermin).

**Methods:** Twenty-one patients (12F and 9M) aged from 21 to 93 years (62.5 ± 19.4) with moderate and severe NK were enrolled in the study. The patients were divided into 2 groups accordingly to the severity of the disease. The underlying causes of NK were determined. The VAS questionnaire was dispensed. The ocular examination comprised slit lamp evaluation, ocular surface assessment with Keratograph 5M (Oculus, Germany), corneal sensitivity with Cochet-Bonnet esthesiometer (Lunneaux, France) and corneal thickness measurement with AC-OCT (DRI, Triton, Topcon, Japan). The drops of Cenegermin 0.002% were administrated 6 times daily for 8 weeks. All participants were evaluated at baseline, after 4 and 8 weeks of the treatment. The area of corneal defect with maximum diameter less than 0.5 mm was considered as healed. The main outcome measures were corneal healing, corneal sensitivity recovery and corneal thickness in the ulcer group.

**Results:** The herpetic keratitis was the most common cause of NK. Thirteen eyes were affected by severe grade of NK with corneal ulcer and 8 eyes presented a moderate grade. After 8 weeks a complete healing of the corneal defects was registered both in moderate and severe NK. Significant increase of the corneal sensitivity and thickness were registered.

**Conclusions:** The rh-NGF (Cenegermin) resulted effective in the treatment of the severe and moderate NK with significant recovery of the corneal sensitivity and healing of the corneal defects in both groups.

## Keywords

Neurotrophic keratitis, rh-NGF, persistent epithelial defects, corneal ulcers, corneal healing, corneal sensitivity

Date received: 26 October 2021; accepted 28 February 2022

## Introduction

The extremely rich corneal innervation originates from the ophthalmic branch of the trigeminal nerve and controls maintenance of the ocular surface homeostasis, ensuring proper functioning of the ocular surface unit and corneal transparency, mandatory for the visual performance.[1] This function is exerted by promoting the release of neurotrophic factors such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3,4 (NT-3, NT-4).[2–6] NGF controls corneal healing and

[1]Ophthalmology Clinic, Department of Biomedical Sciences, University of Messina, Italy
[2]Ophthalmology Department, Andrzej Frycz Modrzewski Krakow University, Poland
[3]IRCCS San Raffaele Scientific Institute, Ophthalmology Clinic, Vita Salute San Raffaele University, Milan, Italy
[4]Department of Economics, University of Messina, Italy

**Corresponding author:**
Anna Maria Roszkowska, Ophthalmology Clinic, University of Messina, Italy, Via Consolare Valeria, 98-125 Messina, Italy.
Email: aroszkowska@unime.it

remodeling through the regulation of epithelial and stromal interaction.[7,8] Reduced level of NGF determines a nerve function impairment and altered corneal function with consequent visual loss.[9,10]

Neurotrophic keratitis (NK) is a degenerative corneal disease resulting from severe impairment of the trigeminal nerve function, causing deficiency of neural factors and the consequent loss of corneal trophism and impaired corneal healing.[9] Nerve malfunction is the hallmark of NK, which manifest clinically in early stage with epithelial defects that not heal.[10] Several factors have been identified as a possible cause of NK such as local or systemic diseases and surgeries, traumatic, infective and metabolic injuries affecting both trigeminal and corneal nerves, being herpetic keratitis the most frequently encountered.[9,11] The disease may present with punctate epitheliopathy, persistent epithelial defects, corneal ulceration and perforation and is accompanied by different grade of impaired corneal sensation.[12] Additionally, low NGF level induces a reduction of tear secretion with consequent ocular surface damage.[13] NK is classified in mild, involving only slight epithelial changes, moderate, with persisting epithelial defects (PED), and severe with different grade of stromal involvement such as ulcer, corneal melting and perforation.[9,12,13] Mastropasqua *et al*. proposed an additional classification based on AC-OCT and in vivo confocal microscopy (IVCM) findings.[14] The diagnosis of NK requires careful investigation of any ocular and systemic condition associated with corneal changes and the testing of corneal sensitivity detecting hypoesthesia is considered as a main finding necessary to confirm diagnosis.[12,13] The therapy is challenging and different therapeutic approaches, both medical and surgical, were adopted. In relation to NK severity different therapies were used such as the therapeutic contact lens (CL), preservative free artificial tears, topical autologous serum, RGTA- a matrix agent mimicking heparan sulfate, thyroxin beta-4, topical substance P, insulin like GF1, amniotic membrane, conjunctival flap, tarsorraphy, neurotization).[15–17] Nevertheless, none of these therapeutic options provided satisfying results.[13,18,19] Lambiase *et al*. demonstrated the effectiveness of murine NGF in promoting corneal healing and sensation recovery in NK patients.[20] Successively, the human recombinant NGF was used and its efficacy in NK was demonstrated in the REPARO 1 and 2 studies.[21,22] Efficacy of rh-NGF was recently confirmed by Pflugfelder *et al*. in a multicenter randomized vehicle-controlled pivotal trial conducted in USA.[23]

The rh-NGF 0.002% (Cenegermin) was approved by European Medicines Agency (EMA) in 2017 and Food and Drug Administration (FDA) in 2018 and actually it represents the first ever topical biologic drug for the first line treatment in patients with NK stage 2 and 3 that not respond to conventional treatment.

In this study we aimed to investigate the clinical and instrumental outcome with morphological and functional corneal recovery in patients with moderate and severe NK treated with Cenegermin. The study was conducted with respect of tenets of Declaration of Helsinki and obtained approval of the Ethical Committee of the University Hospital of Messina.

## Material and methods

Twenty-one patients diagnosed with moderate and severe NK were enrolled in this prospective observational case series study. Twelve patients were female and nine were male and their age ranged from 21 to 93 years (mean 62.5 ± 19.4). (Table 1) All participants were followed in the Ocular Surface diseases section of the Ophthalmology Clinic of the University Hospital of Messina. The exclusion criteria comprised active ocular infection, inflammation not related to NK, peripheral corneal ulcers, bilateral NK involvement and presence of other ocular diseases.

The ocular or systemic pathologies that caused NK were identified. All participants signed the informed written consent to participate in the study. The Visual Analogue Scale (VAS) was used to assess the ocular discomfort at baseline and during the control examinations.

The patients received Cenegermin drops (20 µg/ml) (Oxervate®, Dompè, Farmaceutici Spa, Milan, Italy) accordingly to the standardized protocol with 1 drop for 6 times daily for 8 weeks. (https://www.ema.europa.eu/en/documents/product-information/oxervate-epar-product-information_it.pdf). All subjects were examined at baseline and successively after 4 and 8 weeks at the end of the treatment. The examination included slit lamp biomicroscopy, corneal sensitivity and thickness measurement and ocular surface evaluation. The severity of disease was determined accordingly to Dua *et al*. classification, where the moderate NK was diagnosed in the presence of persistent epithelial defects and the severe stage was determined when the stromal involvement occurred, both associated to corneal hypo or anaesthesia.[12] For the analysis purposes the patients were divided into two groups in relation to the severity of the disease. The first group comprised moderate NK with PED and the second group was composed by the subjects affected by the severe form with corneal ulcers.

Corneal sensitivity was measured at the center of the cornea, with Cochet Bonnet esthesiometer (Lunneaux, France) and expressed in length of file in centimeters (cms), the average value of three measurements was

**Table 1.** Demographic characteristics of the examined patients.

| Patients | Eye | Sex | Age |
|---|---|---|---|
| *21* | *11 Left 10 Right* | *12 F 9 M* | *62.5 ± 19.4* |

recorded for analysis. In the group with ulcer, additionally the corneal thickness was measured with anterior segment module of the Swept Source OCT (AC-OCT, DRI Triton, Topcon, Japan) and the thinnest corneal point, corresponding to the ulcer bed, was considered for evaluation. The corneal surface assessment was done with Keratograph 5M (Oculus, Germany) using the fluorescein staining. The corneas with epithelial defects with maximum diameter lower than 0.5 mm, calculated with Keratograph 5M software, were considered as healed accordingly to REPARO 2.[22]

The statistical analysis was performed using SPSS 26.0 for Windows statistical software (SPSS Inc., Chicago, IL, USA).

**Table 2.** Underlying cause of NK in both studied groups.

| Pathology | Overall | PED | Ulcer |
|---|---|---|---|
| *Herpetic keratitis* | 47.6% | 19% | 28.6% |
| *Post-neuroma surgery* | 19% | 14.3% | 4.7% |
| *Diabetes* | 9.5% | | 9.5% |
| *Surgery* | 4.7% | | 4.7% |
| *Graves' disease* | 4.7% | 4.7% | |
| *Trauma* | 4.7% | | 4.7% |
| *Rheumatoid arthritis* | 4.7% | | 4.7% |
| *Atopic conjunctivitis* | 4.7% | | 4.7% |

A non-parametric approach was adopted for the data analysis using the exact significance of the p value at the 0.05 level. This is due to the small sample size and the results of the Kolmogorov test revealed that the data examined were not normally distributed.

The Friedman test was applied to compare measurements at different times in each group. Wilcoxon's test was used for pair comparisons in each subsequent session.

## Results

All patients completed the therapeutic cycle and no adverse drug effects were recorded during the entire follow-up period. Table 1 reports the clinical characteristics of the examined patients.

The main cause of NK was herpes virus keratitis (47.6%), followed by post-neuroma surgery (19%) and diabetes (9.5%). The remaining causes were previous surgery (4.7%), ocular complications of Graves' disease (4.7%), previous trauma (4.7%), ocular complications of rheumatoid arthritis (4.7%) and atopic conjunctivitis (4.7%). (Table 2)

In 8 eyes the moderate NK with PED was diagnosed while 13 eyes presented a severe form with corneal ulcer.

VAS was $20.2 \pm 6.1$ at baseline, $20.8 \pm 4.7$ at 4 weeks and $8.8 \pm 6.6$ at the end of treatment. It changed

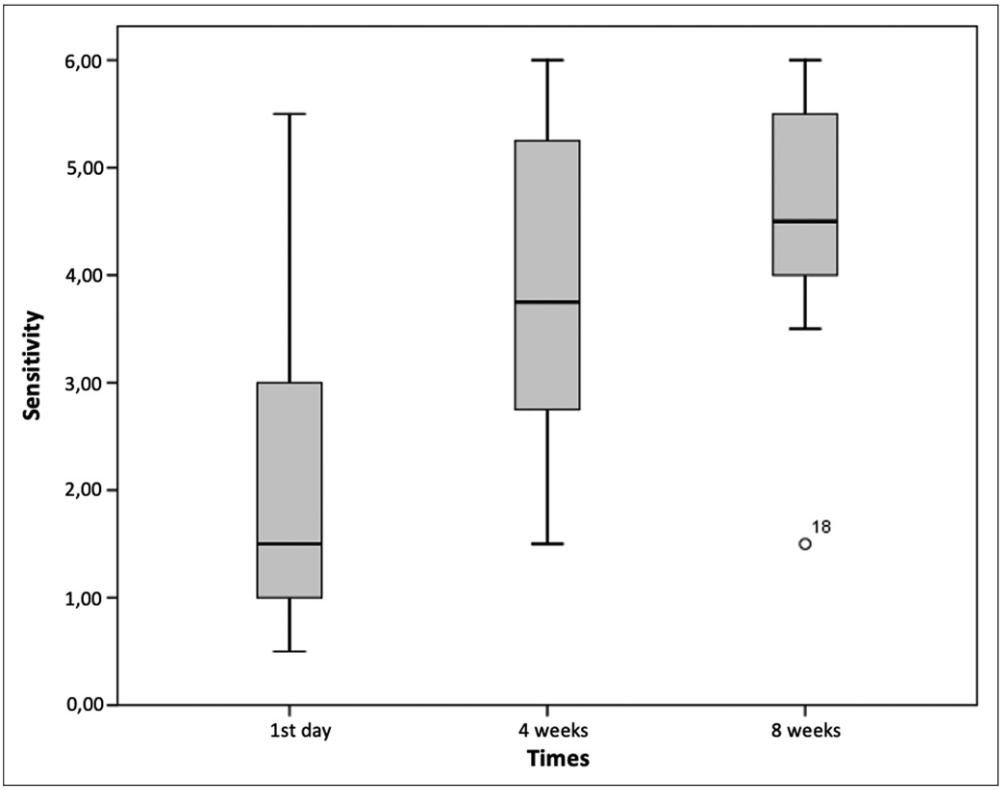


**Figure 1.** Median and inter-quartile range of sensitivity in patients with PED.

significantly when 4 weeks and 8 weeks data were analyzed ($p < 0.001$), while the comparison of baseline and 4 weeks resulted unvaried.

### *Group 1*

*Moderate neurotrophic keratitis (PED).* Moderate stage of NK was present in 8 eyes of 8 patients. In this group the NK originated from herpes virus keratitis in 4 eyes (50%), from post-neuroma surgery in 3 eyes (37.5%) and from ocular complications of Graves' disease in 1 eye (12.5%).

For PED patients there was a statistically difference in corneal sensitivity during the follow up time, ($\chi 2(2) = 11.565$, $p = 0.003$). For this group, a median (IRQ) sensitivity level was: 1.5 (1 to 3.25) at first day, 3.75 (2.375 to 5.375) at 4 weeks and 4.5 (3.75 to 5.50) at 8 weeks. (Figure 1) Post hoc analysis showed the was not a statistically significant differences between 4 weeks and 8 weeks follow-up for sensitivity levels ($p = 0.063$) However, there was a statistically significant increase in sensitivity levels in 4 and 8 weeks in comparison to baseline ($p = 0.031$, $p < 0.032$ respectively).

After 4 weeks treatment the PED's healed in 37.5% (3 of 8) of eyes and at 8 weeks a complete recovery was registered in 100% of eyes (8 of 8). (Figure 2)

### *Group 2*

*Severe neurotrophic keratitis.* Thirteen eyes had severe NK with corneal ulcer. It originated from herpes virus keratitis in 6 eyes (46.1%), from diabetes in 2 eyes (15.3%), from post-neuroma surgery in 1 eye (7.7%), it was post-traumatic in 1 eye, post-surgery in 1 eye (7.7%), related to rheumatoid arthritis in 1 eye (7.7%) and to atopic conjunctivitis in 1 eye (7.7%).

For this group there was a statistically difference in sensitivity during the follow up time, ($\chi 2(2) = 22.37$, $p < 0.001$). Median (IRQ) sensitivity levels at three evaluation times were 1.5 (0,25 to 3.5), 4 (3.5 to 4.75) and 4.5 (3.5 to 5), respectively. (Figure 3) There was a significant increase in sensitivity levels (first day vs 4 weeks $p = 0.001$, first day vs 8 weeks $p = 0.001$ and 4 weeks vs 8 weeks $p = 0.031$). In this group the AC-OCT thickness showed different levels as recorded in the three examination times ($\chi 2(2) = 14.00$; $p = 0.001$). Median (IRQ) OCT levels at baseline, 4 weeks and 8 weeks were 333 (305 to 382), 401 (381 to 423) and 454 (432 to 586), respectively. (Figure 4) The subsequent post hoc analysis showed statistically significant differences in all measurements considered with an increase of corneal stroma as evidenced in AC-OCT pictures ($p = 0.015$ first time vs 4 weeks; $p = 0.016$ first time vs 8 weeks and $p = 0.016$ 4 weeks vs 8 weeks). (Figure 5) After 4 weeks treatment the ulcers healed completely in 69% of eyes (9 of 13) and at 8 weeks a complete recovery was registered in 100% of eyes (13 of 13). (Figure 6)

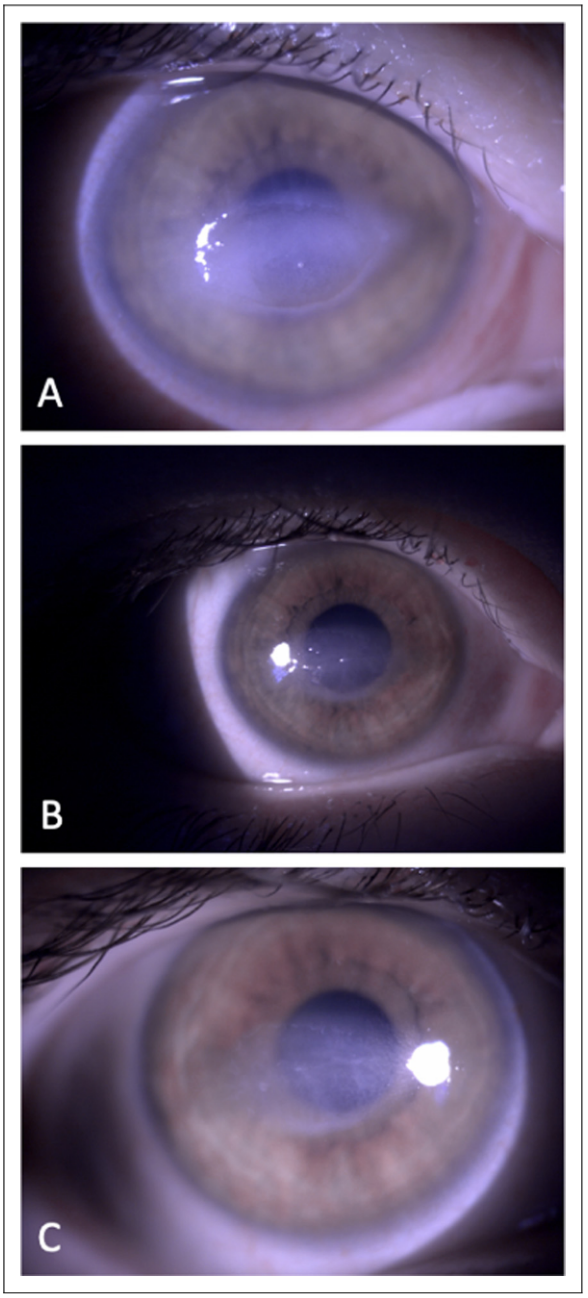


**Figure 2.** Slit lamp images of moderate NK with persistent epithelial defects at baseline (A), after 4 weeks (B) and after 8 weeks (C) treatment with cenegermin. A significant reduction of epithelial defect can be observed.

## Discussion

The injuries to the trigeminal nerve result in the sub-basal nerve plexus (SBNP) fibers damage with consequent altered homeostasis of the ocular surface that negatively impacts on the metabolism and vitality of the corneal epithelium. This might result in different grade of neurotrophic keratitis presenting with epithelial defects, corneal ulceration, melting, and perforation.[9,3] The most

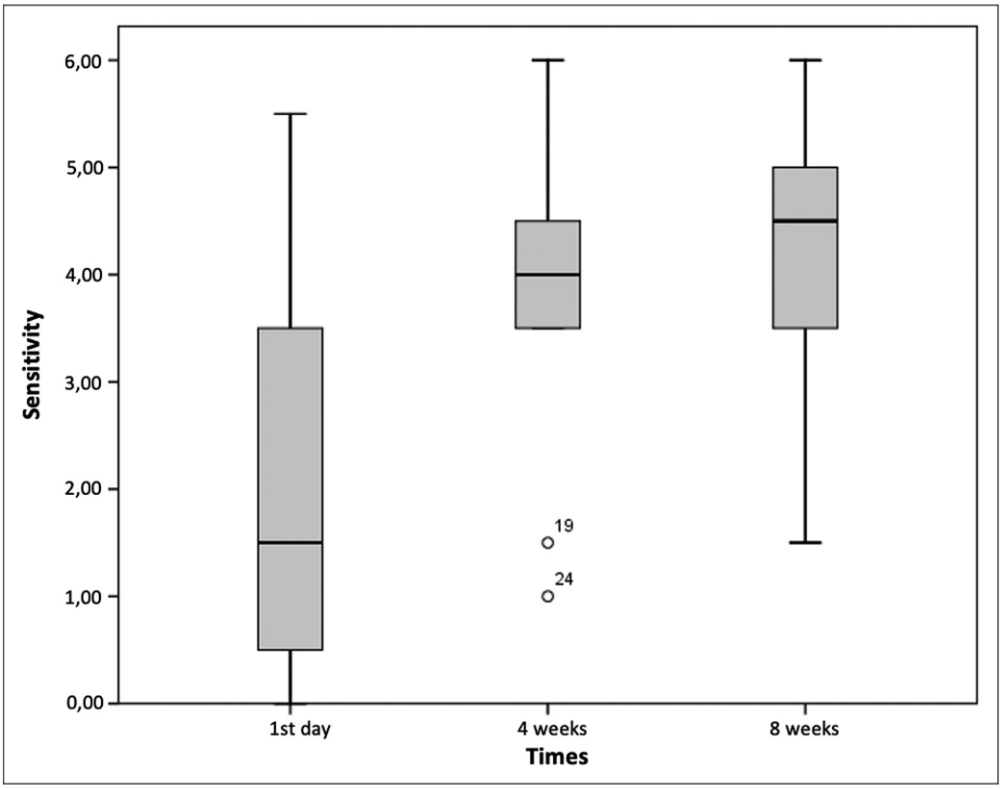


**Figure 3.** Median and inter-quartile range of sensitivity in patients with ulcer.

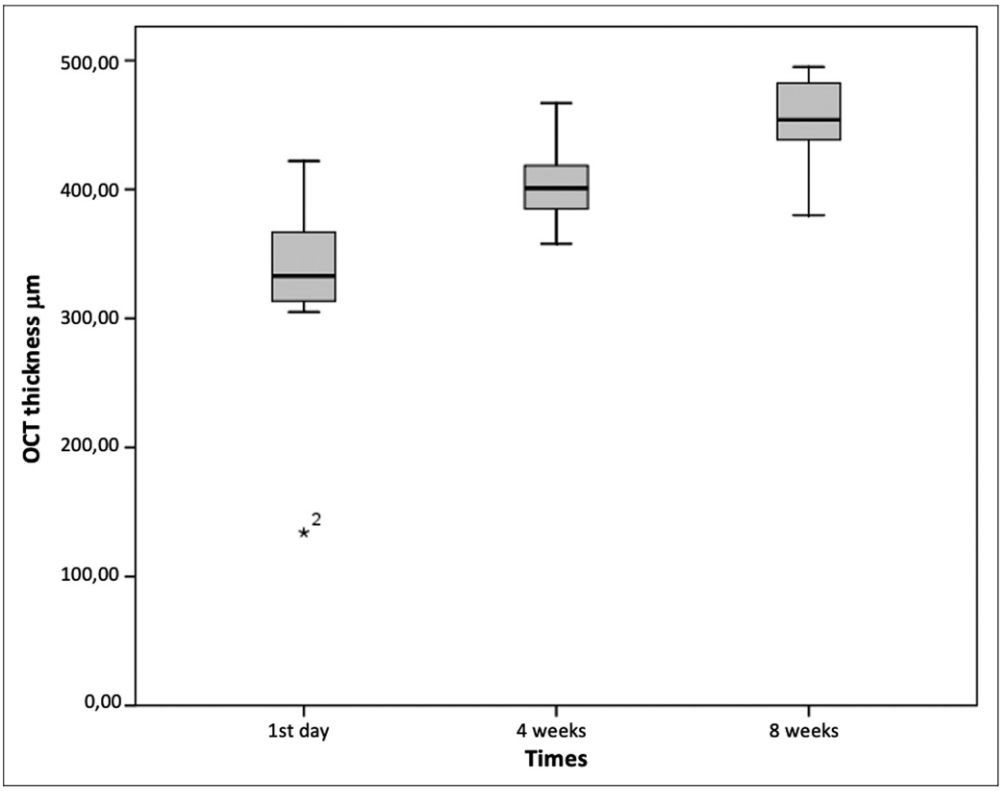


**Figure 4.** Median and inter-quartile range of OCT in the group with ulcer.

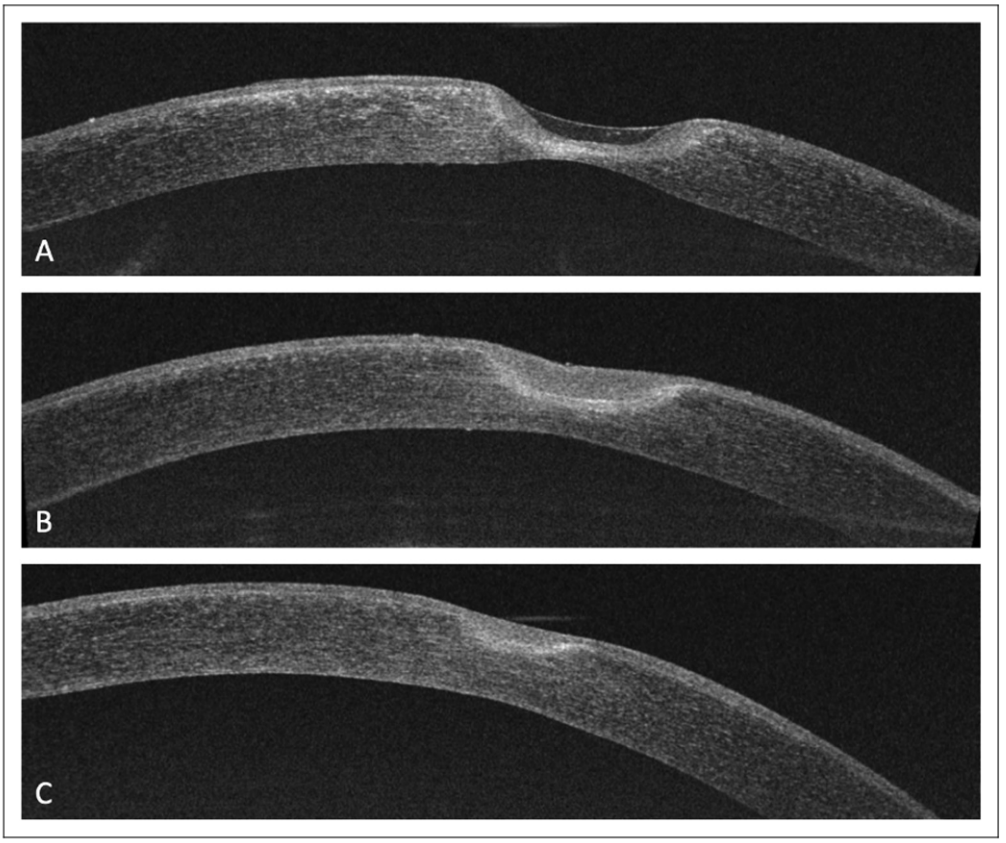


**Figure 5.** AC-OCT pictures of corneal ulcer in severe NK at baseline (A), after 4 weeks (B) and after 8 weeks (C) treatment with cenegermin. Corneal thickness was calculated in the ulcer bed and expressed in microns. Significant increase of the corneal thickness with epithelial integrity restoration after 8 weeks is evident.

important sign of NK is represented by reduced or absent corneal sensitivity that may be assessed through the overall corneal sensitivity testing with simply cotton wisp or accurately quantified with esthesiometers.[24,25]

Different ocular or general conditions such as diabetes mellitus or neurosurgical procedures can induce a V nerve damage, but the herpes virus infection is recognized as a main cause of NK causing the destruction of the sensory ganglion fibers and/or its cell.[12,13,19,26]

In our study the overall main local cause of NK was represented by herpes virus keratitis (47.6%), while diabetes was identified as the major systemic underlying disease with prevalence of 9,5%. These data are in line with those reported in the literature. However, when two groups are considered separately, the diabetes originated NK was observed exclusively in the eyes with severe form involving corneal stroma, and the role of diabetic polyneuropathy in inducing a severe form of NK needs a further investigation.[27] The treatment of NK represents an important challenge and different therapeutic strategies comprising medical and/or surgical approach were reported. The surgical procedures, used mainly for severe cases with ulceration or perforation include tarsorrhaphy, conjunctival flap, lamellar/penetrating keratoplasty and treatment with multilayer amniotic membrane.[28–33] The goal of these treatments is to preserve the anatomical integrity of the eye.[34]

Medical therapies depend on the stage of NK and the ocular condition and include administration of preservative free artificial tears, topical autologous serum, RGTA- a matrix agent, thyroxin beta-4, topical substance P, insulin-like GF1 and different outcomes were reported with frequent clinical failure. None of these substance acts on the nerves trophism that gives the origin of the disease.[35–43]

The use of NGF in the treatment of NK changed the therapeutic rationale as it restores corneal nerves and promotes physiological epithelial healing.[20] Cenegermin is a recombinant form of human nerve growth factor (rhNGF) produced in Escherichia coli, its molecule is identical to human NGF. Preclinical study on different dose of human rhNGF eye drops demonstrated good tolerability and safety of this drug.[44] The clinical studies known as REPARO I and II, demonstrated clinical efficacy and safety of human recombinant NGF (rhNGF) at dose 10 μg/ml and 20 μg/ml in promoting corneal healing in mild and severe NK. In these studies the clinically better outcome was obtained with the higher concentration formulation without changes of

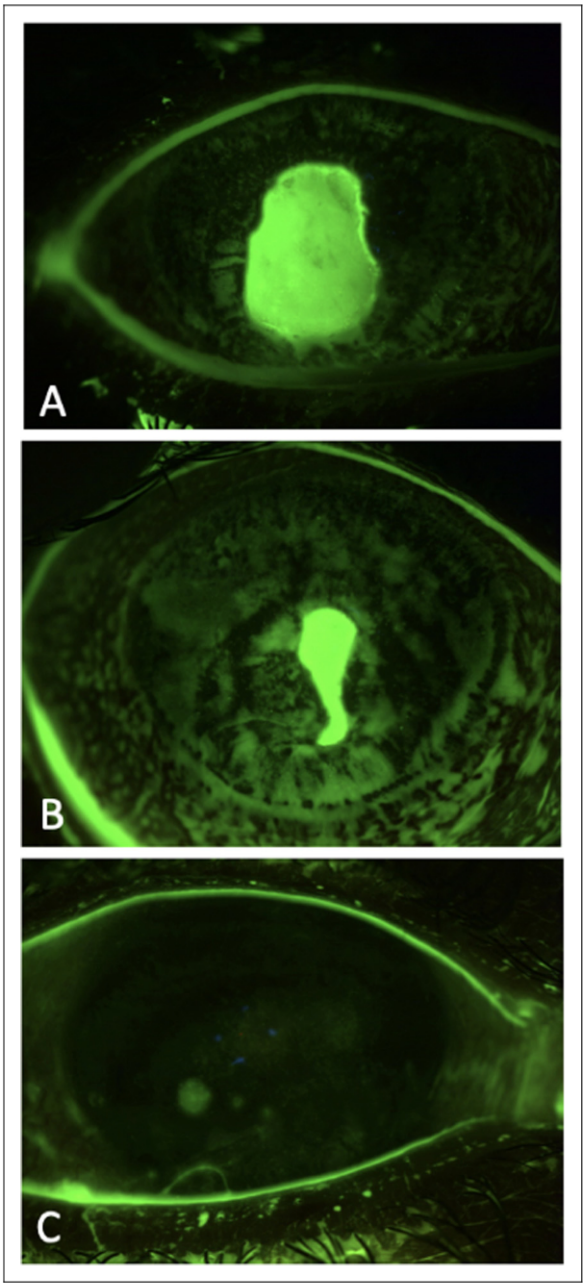


**Figure 6.** Keratograph images of severe NK at baseline (A), after 4 weeks (B) and after 8 weeks (C) treatment with cenegermin. The reduction of the ulcer's area can be observed with a complete epithelial resurfacing at the end of the treatment.

tolerability.[21–22] In 2019 Di Zazzo *et al*. recognized that the treatment with NGF alone leads to improvement of corneal defects.[18] More recently, a study of Mastropasqua *et al*. confirmed the ability of Cenegermin, after 8 weeks of treatment, to heal the persistent epithelial defects and corneal ulcers in a group of 18 patients with NK.[45] A recent report of USA clinical trial using rhNGF 20 µg/ml confirmed the previous findings with encouraging results.[23]

In the present study we have investigated corneal healing in 21 patients with moderate and severe form of NK treated with Cenegermin for 8 weeks accordingly to the administration protocol. We have examined separately the patients with PED and ulcer to identify the eventual differences in healing processes. The grade III (severe) NK was more represented as diagnosed in 13 eyes (61.9%). In group 1 at 4 weeks PED's healed in 37.5% (3 of 8) of eyes and at 8 weeks a complete recovery was registered in 100% of eyes (8 of 8). On contrary, in the group 2, at 4 weeks treatment 69% (9 of 13) of ulcers were closed, while at 8 weeks 100% of eyes were completely healed.

In our study the eyes with severe grade of NK showed better healing and to our knowledge it is the first study that considers separately corneal healing in PED and ulcers. We hypothesized that in ulcers the NGF promotes stromal healing with restore of the corneal thickness that results in faster epithelial healing and surface recovery as compared to PED.

In moderate group the area of defect reduced significantly and it was less than 0.5 mm at the end of the treatment, but the trace staining was still registered showing that PED even if clinically healed, demonstrated worst epithelial recovery when compared to ulcers.

The recovery of corneal sensitivity was observed in both moderate and severe form with significant increment determined after 4 and 8 weeks of the treatment. Corneal sensitivity restore suggests a pivotal role of Cenegermin in the improvement of the corneal trophism.

Additionally, the thickness analysis performed with AC-OCT, in 7 eyes of cooperative patients with ulcers, evidenced a statistically significant differences in all comparisons ($p = 0.015$ baseline vs 4 weeks; $p = 0.016$ baseline vs 8 weeks and $p = 0.016$ 4 weeks vs 8 weeks). As to our knowledge this is the first study that reports morphological data such as changes of the corneal thickness in ulcers.

The overall VAS showed a significant improvement at the end of the treatment but improvement of symptoms was registered after 4 weeks.

These unique data on restoration of the corneal thickness and healing of ulcers after the treatment with Cenegermin confirm the crucial role of NGF in promoting the release of factors involved in maintenance of the integrity of the corneal epithelium and ocular surface hemosthasis.

## Conclusions

The clinical and instrumental data obtained in this study confirm efficacy of the treatment of moderate and severe NK with Cenegermin and provide further information on corneal sensation recovery and thickness restoration. The correct diagnosis and indications are of great importance for the treatment with rh-NGF that proved to promote healing of persistent epithelial defects and ulcers and induce recovery of the corneal sensitivity and trophism.

## Acknowledgements

The authors did not receive any financial support from any public or private sources.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

## ORCID iDs

Anna M Roszkowska https://orcid.org/0000-0002-8083-3437
Pasquale Aragona https://orcid.org/0000-0002-9582-9799

## References

1. Marfurt CF, Cox J, Deek S, et al. Anatomy of the human corneal innervation. *Exp Eye Res* 2010; 90: 478–492.
2. Labetoulle M, Baudouin C, Calonge M, et al. Role of corneal nerves in ocular surface homeostasis and disease. *Acta Ophthalmol* 2019; 97: 137–145.
3. Müller LJ, Marfurt CF, Kruse F, et al. Corneal nerves: structure, contents and function. *Exp Eye Res* 2003; 76: 521–542.
4. Al-Aqaba MA, Anis FS, Mohammed I, et al. Nerve terminals at the human corneoscleral limbus. *Br J Ophthalmol* 2018; 102: 556–561.
5. Shaheen BS, Bakir M and Jain S. Corneal nerves in health and disease. *Surv Ophthalmol* 2014; 59: 263–285.
6. You L, Kruse FE and Völcker HE. Neurotrophic factors in the human cornea. *Invest Ophthalmol Vis Sci* 2000; 41: 692–702.10711683
7. Chen L, Wei RH, Tan DT, et al. Nerve growth factor expression and nerve regeneration in monkey corneas after LASIK. *J Refract Surg* 2014; 30: 134–139.
8. Di G, Qi X, Zhao X, et al. Corneal epithelium-derived neurotrophic factors promote nerve regeneration. *Invest Ophthalmol Vis Sci* 2017; 58: 4695–4702.
9. Bonini S, Rama P, Olzi D, et al. Neurotrophic keratitis. *Eye (Lond)* 2003; 17: 989–995.
10. Araki K, Ohashi Y, Kinoshita S, et al. Epithelial wound healing in the denervated cornea. *Curr Eye Res* 1994; 13: 203–211.
11. Semeraro F, Forbice E, Braga O, et al. Evaluation of the efficacy of 50% autologous serum eye drops in different ocular surface pathologies. *Biomed Res Int* 2014; 2014: 826970.25136628
12. Dua HS, Said DG, Messmer EM, et al. Neurotrophic keratopathy. *Prog Retin Eye Res* 2018; 66: 107–131.
13. Sacchetti M and Lambiase A. Diagnosis and management of neurotrophic keratitis. *Clin Ophthalmol* 2014; 8: 571–579.24672223
14. Mastropasqua L, Nubile M, Lanzini M, et al. In vivo microscopic and optical coherence tomography classification of neurotrophic keratopathy. *J Cell Physiol* 2019; 234: 6108–6115.
15. Donnerer J, Schuligoi R and Stein C. Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. *Neuroscience* 1992; 49: 693–698.
16. Brown SM, Lamberts DW, Reid TW, et al. Neurotrophic and anhidrotic keratopathy treated with substance P and insulin-like growth factor 1. *Arch Ophthalmol* 1997; 115: 926–927.
17. Tripathi BJ, Kwait PS and Tripathi RC. Corneal growth factors: a new generation of ophthalmic pharmaceuticals. *Cornea* 1990; 9: –9.
18. Di Zazzo A, Varacalli G, Mori T, et al. Long-term restoration of corneal sensitivity in neurotrophic keratopathy after rhNGF treatment. *Eur J Ophthalmol* 2020; 27: 1120672120953343.
19. Versura P, Giannaccare G, Pellegrini M, et al. Neurotrophic keratitis: current challenges and future prospects. *Eye Brain* 2018; 10: 37–45.
20. Lambiase A, Rama P, Bonini S, et al. Topical treatment with nerve growth factor for corneal neurotrophic ulcers. *N Engl J Med* 1998; 338: 1174–1180.
21. Bonini S, Lambiase A, Rama P, et al. REPARO study group. Phase I trial of recombinant human nerve growth factor for neurotrophic keratitis. *Ophthalmology* 2018; 125: 1468–1471.
22. Bonini S, Lambiase A, Rama P, et al. REPARO study group. Phase II randomized, double-masked, vehicle-controlled trial of recombinant human nerve growth factor for neurotrophic keratitis. *Ophthalmology* 2018; 125: 1332–1343.
23. Pflugfelder SC, Massaro-Giordano M, Perez VL, et al. Topical recombinant human nerve growth factor (Cenegermin) for neurotrophic keratopathy: a multicenter randomized vehicle-controlled pivotal trial. *Ophthalmology* 2020; 127: 14–26.
24. Norn MS. Measurement of sensitivity. In: Norn MS (ed) *External eye diseases. Methods of examination*. Copenhagen, Denmark: Munksgaard International Publisher Ltd, 1974.
25. Belmonte C, Acosta MC, Schmelz M, et al. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO2 esthesiometer. *Invest Ophthalmol Vis Sci* 1999; 40: 513–519.
26. Hamrah P, Cruzat A, Dastjerdi MH, et al. Corneal sensation and subbasal nerve alterations in patients with herpes simplex keratitis: an in vivo confocal microscopy study. *Ophthalmology* 2010; 117: 1930–1936.
27. Lockwood A, Hope-Ross M and Chell P. Neurotrophic keratopathy and diabetes mellitus. *Eye (Lond* 2006; 20: 837–839.
28. Portnoy SL, Insler MS and Kaufman HE. Surgical management of corneal ulceration and perforation. *Surv Ophthalmol* 1989 Jul-Aug; 34: 47–58.
29. Lugo M and Arentsen JJ. Treatment of neurotrophic ulcers with conjunctival flaps. *Am J Ophthalmol* 1987; 103: 711–712.
30. Lee SH and Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol* 1997; 123: 303–312.
31. Khokhar S, Natung T, Sony P, et al. Amniotic membrane transplantation in refractory neurotrophic corneal ulcers: a

randomized, controlled clinical trial. *Cornea* 2005; 24: 654–660.
32. Chen HJ, Pires RT and Tseng SC. Amniotic membrane transplantation for severe neurotrophic corneal ulcers. *Br J Ophthalmol* 2000; 84: 826–833.
33. Kruse FE, Rohrschneider K and Völcker HE. Multilayer amniotic membrane transplantation for reconstruction of deep corneal ulcers. *Ophthalmology* 1999; 106: 1504–1510.
34. Mackie IA. Neuroparalytic keratitis. In: Fraunfelder FT, Roy FH and Grove J (eds) *Current ocular therapy*. 4th ed. Philadelphia: W. B. Saunders, 1995, pp.452–454.
35. Turkoglu E, Celik E and Alagoz G. A comparison of the efficacy of autologous serum eye drops with amniotic membrane transplantation in neurotrophic keratitis. *Semin Ophthalmol* 2014; 29: 119–126.
36. Matsumoto Y, Dogru M, Goto E, et al. Autologous serum application in the treatment of neurotrophic keratopathy. *Ophthalmology* 2004; 111: 1115–1120.
37. Quinto GG, Campos M and Behrens A. Autologous serum for ocular surface diseases. *Arq Bras Oftalmol* 2008; 71: 47–54.
38. Lopez-Plandolit S, Morales MC, Freire V, et al. Plasma rich in growth factors as a therapeutic agent for persistent corneal epithelial defects. *Cornea* 2010; 29: 843–848.
39. Jeng BH and Dupps WJJr. Autologous serum 50% eyedrops in the treatment of persistent corneal epithelial defects. *Cornea* 2009; 28: 1104–1108.
40. Rao K, Leveque C and Pflugfelder SC. Corneal nerve regeneration in neurotrophic keratopathy following autologous plasma therapy. *Br J Ophthalmol* 2010; 94: 584–591.
41. Aifa A, Gueudry J, Portmann A, et al. Topical treatment with a new matrix therapy agent (RGTA) for the treatment of corneal neurotrophic ulcers. *Invest Ophthalmol Vis Sci* 2012; 53: 8181–8185.
42. Guerra M, Marques S, Gil JQ, et al. Neurotrophic keratopathy: therapeutic approach using a novel matrix regenerating agent. *J Ocul Pharmacol Ther* 2017; 33: 662–669.
43. Dunn SP, Heidemann DG, Chow CY, et al. Treatment of chronic nonhealing neurotrophic corneal epithelial defects with thymosin beta4. *Ann N Y Acad Sci* 2010; 1194: 199–206.
44. Ferrari MP, Mantelli F, Sacchetti M, et al. Safety and pharmacokinetics of escalating doses of human recombinant nerve growth factor eye drops in a double-masked, randomized clinical trial. *BioDrugs* 2014; 28: 275–283.
45. Mastropasqua L, Lanzini M, Dua HS, et al. In Vivo Evaluation of Corneal Nerves and Epithelial Healing After Treatment With Recombinant Nerve Growth Factor for Neurotrophic Keratopathy. *Am J Ophthalmol*. 2020; 217: 278–f286.