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Original article

How long can *Varroa destructor* survive on its host *Apis mellifera*?

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Abstract – *Varroa destructor* is the major ectoparasite of honey bees causing extensive colony losses worldwide. Despite the importance of this threat to honey bees some crucial aspects of the mite's biology are still poorly known, including the longevity of the parasite on the different developmental stages of its host. This study aimed at determining, under standard conditions, the lifespan of *V. destructor* on the larvae, pupae, and adults of the honey bee. In our study, *V. destructor* survival varied depending on the host life stage, with the longest survival observed on honey bee larvae, where median and maximum survival up to 45 and 100 days were recorded, respectively. A declining survival of *V. destructor* during the summer season was also noted, possibly linked to deteriorating honey bee health, associated with higher viral infection levels. This study emphasizes the importance of establishing an artificial rearing system to advance research on *V. destructor* biology and facilitate the development of innovative control strategies.

***Varroa destructor* / Lifespan / *Apis mellifera* / Artificial rearing / Mite**

1. INTRODUCTION

Varroa destructor Anderson & Trueman (Parasitiformes: Varroidae) is the most important ectoparasite of the Western honeybee (*Apis mellifera* L.). This mite is considered, together with the pathogenic deformed wing virus (DWV), that it is mainly vectored by *V. destructor*, the major responsible of the extensive colony losses recorded in the northern hemisphere in the last decades (Genersch et al. 2010; Neumann and Carreck 2010; Smith et al. 2013; Nazzi and Le Conte 2016). Notably, despite the relevance of this biotic threat to honey bee health, there is still a serious lack of knowledge regarding many fundamental aspects of *V. destructor* biology (Traynor et al. 2020). Longevity is a crucial feature of the life cycle of any organism and a critical parameter to describe its population dynamics. Nevertheless, the longevity of *V. destructor*, which is linked to the potential number of reproductive cycles the mite can perform, has not yet been defined with sufficient confidence (Fries et al. 1994; Reams and Rangel 2022). As a matter of fact, the existing models predicting *V. destructor* population dynamics are based on many assumptions on daily mortality rate and the average number of reproductive cycles (Fries et al. 1994; Martin 1998; CaLis et al. 1999; DeGrandi-Hoffman and Curry 2004).

Available data have been collected both under natural (Table I) and artificial conditions (Table II) by many authors since *V. destructor* first appeared in the western world. In general, longer survival

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Table I. Previous data on the lifespan of *V. destructor* under natural conditions

Authors	Methods	Mean/max lifespan	Notes
Shabanov et al. (1978)	Reporting data from other unspecified authors	2–3 months in Summer 5–8 months in Autumn	Origin of data unknown
Smirnov (1978)	Reporting data from Lange (1976)	2 months in Summer 5 months in Winter	Origin of data unknown
Schulz (1984)	Marked mites were inserted into the hive and observed during Summer	The author reports that a mite can wait up to 50 days between one reproductive cycle and another	Many mites got lost
de Ruijter (1987)	Adult female mites were artificially introduced inside recently sealed brood cells for successive cycles	Maximum lifespan: 2.5 months	Most of the mites used in the experiment had disappeared by the end of it
Calatayud and Verdú (1994)	Mites falling on bottom boards were counted at 3–4 days interval for 3 months in broodless colonies	Mean life expectancy: 31 days Maximum lifespan: 80–100 days	Only phoretic mites were considered

data were reported under natural conditions but methodological details are often missing. On the other hand, a notable variability can be recognized among data collected under the more standardized lab conditions, with some authors indicating a maximum lifespan around one month (e.g. Egekwu et al. 2018; Posada-Florez et al. 2020) and others reporting values as long as two–three months under lab conditions (e.g. Ryabov et al. 2022).

Robust data regarding the longevity of *V. destructor* is critical not only for modeling purposes but also to establish a keystone to evaluate ongoing attempts to develop an effective artificial rearing system for the parasite (Piou et al. 2023). This is an important pre-requisite for the development of novel control methods against *V. destructor* (Vilarem et al. 2021); moreover, reliable data about *V. destructor* longevity would allow in turn the clarification of many other aspects of the mite's biology. For example, recently, Ramsey et al. (2019) claimed that the mite feeds exclusively on the honey bee's fat body. Their conclusion is also based on the fact that reproducing mites feeding on a diet largely composed of fat body survived 3.5 ± 1.5 days as compared to mites receiving a diet containing hemolymph only which, instead, survived 1.8 ± 0.8 days. The biological relevance of this difference can be judged only with respect to the duration of the mite's life. In fact, if the longevity of *V. destructor* were on average 30 days, as available data seem to suggest, then we could, at most, state that mites feeding on a fat body rich diet are dying slightly slower than the others, since the longevity of mites feeding on the presumably best diet is only one tenth of normal.

Therefore, to establish how long *V. destructor* can survive on its host, we compared the longevity of mites of known physiological conditions (at the beginning of the reproductive phase, 0–15 h after cell sealing) on their natural host, under standardized environmental conditions (34.5 °C and 80% R.H.). An experiment with bee larvae as the only feeding source was carried out in 2022 and a more complete experiment with larvae, pupae and adult bees as a feeding source was performed in 2023. In all experiments, *V. destructor* survival on its living host was monitored daily.

We hope that, besides contributing to fill a longstanding gap regarding the biology of *V. destructor*, this data will serve as a positive control for future studies aiming at the artificial rearing of this ectoparasite.

Table II. Previous data on the lifespan of *V. destructor* under laboratory conditions on the living host

Authors	Methods	Mean/max lifespan	Notes
Mikityuk et al. (1976)	T = 33–35 °C, R.H. = 86%, mites were kept on bee brood	- Mean lifespan changed over the season (from May to October) - Longer mean lifespans (i.e. 24.2 days) were recorded in July	Some missing information on how the experiment was conducted
Avdeeva (1978)	T = 34–35 °C, R.H. = 74–93%, mites were kept on bee larvae changed every 12 days	Maximum lifespan: 78 days	Median survival not reported
Accorti and Nannelli (1988)	T = 35 °C, R.H. = 85%, mites were kept on bee larvae changed every 8–13 days	- Mean lifespan: 25.1 days - Maximum lifespan: 81 days	The experiment is not clearly explained
Chiesa and Milani (1988)	T = 34 °C, R.H. = 85%, mites were kept on bee larvae changed every 7–12 days	Median survival: 25 days	Maximum survival not reported
Milani and Chiesa (1989)	T = 34.5 °C, R.H. = 85%, mites were kept on bee larvae changed every 3 days	Median survival: more than 30 days	Data on survival reported only until day 30
Egekwu et al. (2018)	T = 32.5 °C, R.H. = 85%, mites kept in gelatine capsules on bee pupae changed every 6 days	- Median survival: 9–13 days - Mean lifespan: 12–14 days - Max lifespan: 33 days	
Posada-Florez et al. (2020)	T = 32.1 °C, R.H. = 82.2%, mites were kept on bee pupae changed every 3–4 days	- Median lifespan: 11 days - Mean lifespan: 9.42 days - Max lifespan: 25 days	
Ryabov et al. (2022)	T = 33 °C, R.H. = 85%, mites were in kept in gelatine capsules on bee pupae changed every 6 days	- Median lifespan: 19 days - Mean lifespan: 20.9 days - Max lifespan: 75 days	- No seasonal trend - Mean and maximum lifespan calculated from supplementary material

2. MATERIALS AND METHODS

2.1. Biological material

The mites and the bees used in the experiments were obtained from 10 hives located in the experimental apiary of the University of Udine (Italy, 46°04′53.3″ N, 13°12′33.1″ E). The local bee population is a natural hybrid between *A. m. ligustica* and *A. m. carnica* (Comparini and Biasolo 1991; Nazzi 1992).

Worker bee larvae and female *V. destructor* mites were collected from brood cells that had been capped in the preceding 15 h, according to Chiesa and Milani (1988). As it is not possible to assess the age of mites at the time of sampling, we used mites collected from the hive during this time window to ensure that they were homogenous under the physiological point of view (i.e. at the same time point in their life cycle).

2.2. Longevity of *V. destructor* on different life stages of the host

To establish the longevity of *V. destructor* on the different life stages of its host, mites were randomly divided into four groups. Two groups of mites were placed in polystyrene sterile Petri dishes (ø = 6 cm, Sigma-Aldrich) on bee larvae. Each Petri dish contained five 5th instar (L5) honey bee larvae, recently sealed, and five mites. Mites could freely move from one host to another. Mites from larvae group (L), were allowed to feed exclusively upon hosts at the larval stage. This was achieved by replacing the original L5 larvae with new L5 larvae, every 3–4 days. Mites from pupae group (P) were allowed to feed on honey bees at the pupal stage. This was achieved by replacing the original L5 larvae with new L5 larvae, every 10–12 days (i.e. upon completing the metamorphosis), starting from recently sealed L5. Mites from control group (Ctrl) were placed in Petri dishes without any host. Mites from adults group (A) were carefully placed with a fine brush on newly emerged worker bees coming from the same apiary. Before the artificial infestation, the newly emerged bees were carefully inspected for the presence of *V. destructor* and only mite-free bees were used. The mites (1 mite/bee) and adult bees (n = 25) were then placed inside plastic cages (18 x 10 x 9 cm, provided by a local supplier). Adult bees were fed with water and a sugar solution (61% glucose, 39% fructose; Thom et al. 2003) provided through two 20 mL syringes. Bees and mites from all the experimental groups were kept in a climatic chamber (34.5 °C, 80% R.H.). These conditions were chosen based on previous work (Sakai et al. 1979; Accorti and Nannelli 1988; Chiesa et al. 1989; Garedew et al. 2004; Jack et al. 2020; Johnson et al. 2024) to achieve the best compromise between *V. destructor* survival and the proper development of the host. Moreover, the temperature in the brood area of the hive is usually maintained between 32 and 36 °C with a mean of 34.5 °C (Becher et al. 2010). Therefore, the temperature we used in our experiments is coherent with the one found inside the hive, an important pre-requisite in the study of a parasite rarely leaving the nest of honey bees.

The Petri dishes and the experimental cages were inspected daily to assess the survival of mites. Overall, the experiment was replicated four times: in June, in July (two times) and in September 2023, with a total of 125, 130, 130 and 100 mites used, respectively.

The same experiment but with one experimental group only (L: larvae) was carried out in 2022. Overall, the experiment was replicated three times; in July, August and September, with a total of 23, 50 and 35 mites, respectively.

2.3. Seasonal decline of the host's conditions

To assess the importance of the conditions of the bee larvae as changing through the season, a convenient number of L5 larvae, obtained as explained above, were maintained in gelatine capsules according to Nazzi and Milani (1994). To this purpose, with the help of a fine paint brush, one honey bee larva was manually inserted into the bottom half of a 6.5 mm gelatine capsule (size 1, Agar Scientific Ltd). The capsule was then closed with its lid that had been punctured three times with an entomological needle for ventilation purposes. The larvae were maintained at 34.5 °C, 80% R.H. for 11 days, then the number of bees that reached the adult stage was counted. The ratio between the number of adult bees counted on the 11th day and the initial number of larvae was used to calculate the percentage of surviving bees. The experiment was repeated four times in August and September 2023, with a total of 290, 350, 300 and 205 bees.

2.4. Statistical analysis

Survival curves were built using the Kaplan–Meier procedure with the software

GraphPad Prism 10.2.0. During the follow-up period, individuals were censored if lost or dead due to technical problems. A censored individual was considered alive until the day of censoring. Censored data in survival curves refer to instances where the exact survival time of an individual is unknown, often because the individual was lost to follow-up. In survival analysis, these cases are marked as censored, indicating that the data is incomplete but still valuable for analysis. The survival curves were compared using the Log-rank test using the same software. Survival curves of mites maintained on different honey bee life stages (i.e. L, P, A and Ctrl) were compared with each other, within each of the four replicates of the experiment.

Medians were used as the descriptive statistic in our survival analysis because they are less affected by outliers, skewed and censored data. In particular, the distribution of survival times can be highly skewed, with some individuals surviving much longer than others; these extreme values can distort the mean, making it unrepresentative of survival time; instead, in such cases, the median provides a more robust measure of central tendency (Schober and Vetter 2018).

The results of the experiment on the seasonal decline of the host's conditions were compared using the Fisher's exact test. In this case, the percentage of bees reaching the imago stage was used as a descriptor, but the analysis is based on the number of normally developed newly emerged bees versus the number of bees dead before eclosion.

Whenever multiple comparisons were carried out, type I errors were prevented using the Bonferroni correction. To assess significance, probabilities were compared to 0.05/n, where n corresponds to the number of comparisons. Only statistically significant results were reported.

3. RESULTS

3.1. Longevity of *V. destructor* on different life stages of the host

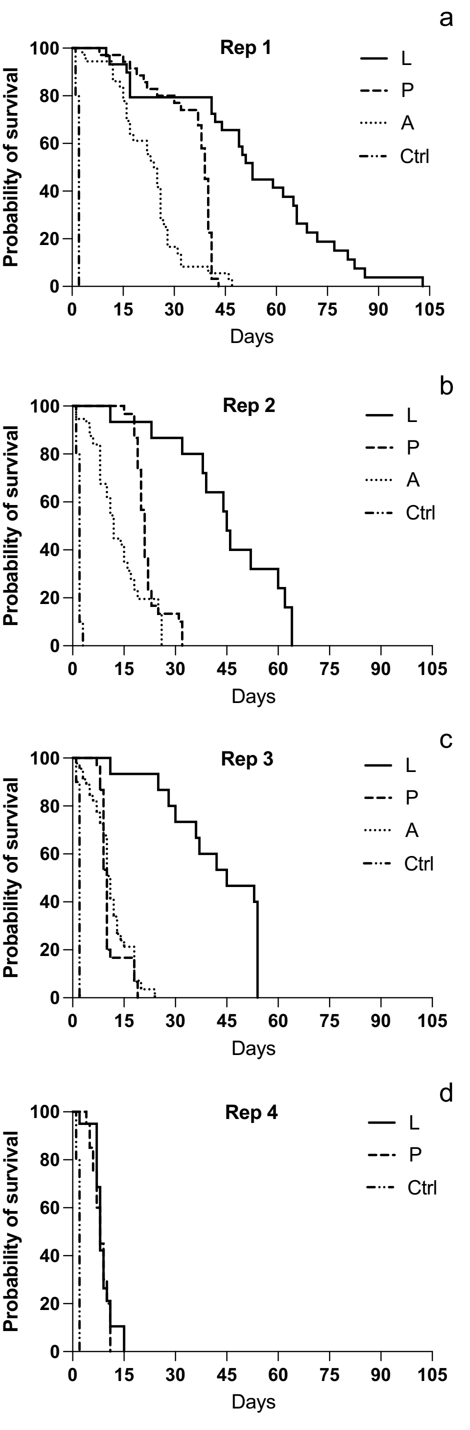
The life stage of the host significantly influenced the lifespan of *V. destructor* (Figure 1, Table III). In fact, the survival of mites feeding on honey bee larvae was significantly longer than that of mites feeding on pupae and adults in all replicates except the fourth one (rep 1: L vs P: $\chi^2 = 26.68$, df = 1, $P < 0.0001$, L vs A: $\chi^2 = 35.18$, df = 1, $P < 0.0001$; rep 2: L vs P: $\chi^2 = 28.58$, df = 1, $P < 0.0001$, L vs A: $\chi^2 = 22.02$, df = 1, $P < 0.0001$; rep 3: L vs P: $\chi^2 = 36.75$, df = 1, $P < 0.0001$, L vs A: $\chi^2 = 32.85$, df = 1, $P < 0.0001$; Figure 1, Table III, Table IV and the survival of mites feeding on pupae was significantly longer than that of mites feeding on adult bees in replicate 1 and 2 (rep 1: P vs A: $\chi^2 = 15.78$, df = 1, $P < 0.0001$; rep 2: P vs A: $\chi^2 = 13.60$, df = 1, $P = 0.0002$; Figure 1, Table III, Table IV). On the host's life stage allowing the longest survival (i.e. the larval stage), mites survived up to 103 days (Figure 1a, Table III) with a median survival of 53 days (Table III). To our knowledge, this represents the longest lifespan ever observed under laboratory conditions (Table II) and approaches previous data obtained under natural conditions (Table I). Conversely, starving *V. destructor* mites had a maximum survival of 3 days and a median lifespan of 2 days (Figure 1, Table III).

A clear seasonal trend in *V. destructor* survival was observed. In particular, the median survival markedly decreased along the season especially in mites kept on larvae and pupae (Figure 1, Table III). The decline of the median survival along the season noted in 2023 mirrored that observed in a preliminary experiment carried out in 2022 with mites maintained on L5 larvae only (Figure 2, Table V).

3.2. Seasonal decline of the host's conditions

In order to test whether the observed seasonal decline of mite survival depended on the conditions of the parasite or that of the host, we assessed the survival of L5 honey bee larvae maintained under artificial conditions until emergence. The percentage of bees reaching the adult stage after 11 days in gelatine capsules varied from nearly 90% in August to 50% one

Figure 1. Kaplan–Meier survival curves of mites maintained▶ on three life stages of honey bees (L: larvae, P: pupae, A: adults) and without host (Ctrl), on four different dates in 2023: **a** 22nd of June; **b** 13th of July; **c** 20th of July; **d** 4th of September.



month later (Table VI). The difference between the first replicate (11th of August) and the third (1st of September) as well as the fourth (8th of September) was highly significant ($P < 0.0001$ in both cases) (Fisher's exact test). The second replicate (25th of August) does not differ significantly from the first one ($P = 0.15$) but the difference is highly significant compared to the third and fourth replicate ($P < 0.0001$ in both cases) (Fisher's exact test).

4. DISCUSSION

Under standardized conditions, *V. destructor* median survival on its host was longer than 45 days, with some mite living longer than 100 days, confirming previous results (Table I, Table II). The longer mites' survival observed on bee larvae as compared to that on pupae and adult bees matches the results obtained by Piou et al. (2023) who observed a greater longevity when larval haemolymph was provided to mites, as compared to the survival of mites receiving pupae or adult bees' haemolymph. Larval cuticle is easier to perforate by the mite because it is less sclerotized (Hopkins and Kramer 1992) which would imply that less energy is spent by the mite for the feeding process. Moreover, larvae normally have a larger relative haemolymph volume than adults (Kanost 2009). *V. destructor* does not have particularly strong muscles connected to the perforating apparatus or to the pharyngeal pump (Akimov and Yastrebtsov 1983; Griffiths 1988; Li et al. 2019), therefore a thinner cuticle and a larger volume of liquid would highly facilitate the feeding process of the mite. Furthermore, the composition of the haemolymph differs between larval, pupal and adult stages (Milani 1988; Chan et al. 2006; Dereje et al. 2013; Ghosh et al. 2016) and a higher nutritional value of larval haemolymph is therefore plausible. Regarding

Table III. Number of mites used in each replicate (I, II, III, IV) of the experiment carried out in 2023, median and maximum survival. A subject was censored when it was lost or if its dead was due to technical problems of the experiment. N/A means not applicable

	Number of mites				Median survival (days)				Maximum survival (days)			
	I (22nd of June)	II (13th of July)	III (20th of July)	IV (4th of September)	I	II	III	IV	I	II	III	IV
L	30	15	15	20	53	45	45	8	103	64	54	15
P	35	30	30	20	39	21	10	8	43	32	19	11
A	50 (14 censored)	75 (36 censored)	75 (36 censored)	50 (all censored)	24.5	12	11	N/A	47	24	18	N/A
Ctrl	10	10	10	10	2	2	2	2	2	3	2	2

Table IV. Significance of the difference between treatments (L: larvae, P: pupae, A: adults) for each replicates (I, II, III, IV). Three asterisks mark significant differences between treatments at $P < 0.001$; ns non significant, N/A not applicable

	Replicate			
	I	II	III	IV
L vs P	***	***	***	ns
L vs A	***	***	***	N/A
P vs A	***	***	ns	N/A

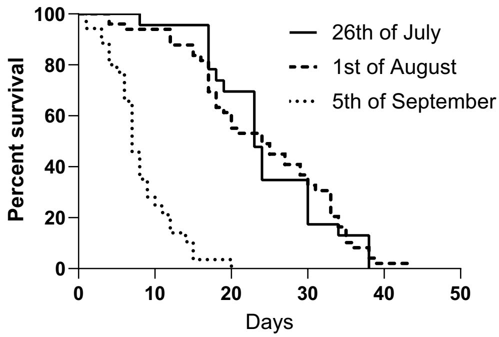


Figure 2. Kaplan–Meier survival curves of *V. destructor* maintained on honey bee larvae (L) in 2022 (plain line: 26th of July; dashed line: 1st of August; dotted line: 5th of September).

the comparison of *Varroa* mite survival between the three honey bee life stages considered in this study, it must be noted that, in general, the assessment of mite survival on adult bees poses a series of practical issues that were also encountered by other authors (see Table I). Similarly, as the season progressed the proportion of decaying pupae became a serious issue.

Under the practical point of view, this study demonstrates that, despite the great effort and the many attempts carried out so far, our ability to rear the *Varroa* mite on artificial substrates (without the living host) is still limited, in that the longest median survival obtained under these conditions so far is 23 days (Piou et al. 2023). Furthermore, the results reported here support the reconsideration of a recent study regarding

Table V. Number of mites used in each replicate of the experiment carried out in 2022, median and maximum survival

Group	Date	Number of mites	Median survival (days)	Maximum survival (days)
1st replicate	26th of July	23	23	38
2nd replicate	1st of August	50	24	43
3rd replicate	5th of September	35	7	20

Table VI. Number of L5 honeybee larvae used and percentage of bees reaching the adult stage after 11 days in gelatine capsules under controlled conditions in each replicate of the experiment related to the seasonal decline of the host's conditions

Starting date of the experiment	Number of bees used in the experiment	Percentage of bees reaching the adult stage
11th August	290	89.7
25th August	350	85.7
1st September	300	68.3
8th September	205	50.7

V. destructor biology (Ramsey et al. 2019). In particular, the idea that a survival as long as 3.5 days on a fat body rich diet can be regarded as a convincing proof of the fact that the mite feeds on that pabulum and not haemolymph must be reconsidered in view of the fact that, on a convenient diet, the mite can survive 30 times longer. It is worth noting that, in our experiment, mites with no access to food lived up to 3 days, with a median survival of 2 days (Table III). This data is in accordance with the average survival of mites without any source of food reported by de Guzman et al. (1991) and approaches the average survival reported by Ramsey et al. (2019) on a fat body diet.

The presumed preference of the mite for feeding on the honey bee's fat body is further challenged by the longer lifespan of mites feeding on larvae than adults, that was obtained in this study and already observed by Piou et al. (2023). As a matter of fact, at the larval stage, fat body cells are dispersed throughout the haemolymph and are not organized into a single organ (Dade 2009); therefore, at this stage, it would be impossible for *V. destructor* to feed exclusively on the fat body, as stated by Ramsey et al. (2019). Recent findings by Han et al. (2024) demonstrated that *V. destructor* of all ages mainly consume haemolymph when feeding on pre-imaginal honey bee stages and notoriously *V. destructor* feeds much less on adult bees than on pre-imaginal stages (de d'Aubeterre et al. 1999; Erban et al. 2015).

The decline in the survival observed in 2023 from the end of July parallels the deteriorating health conditions of honey bees because of the increasing viral infection levels reported in this geographical area around the same time of the year (Nazzi et al. 2012; Breda et al. 2022). This interpretation is confirmed by our results regarding the percentage of larvae developing into adults according to the time of season (Table VI). On the other hand, this explanation does not exclude the possibility that the changes in the mite's lifespan are due to the conditions of the mite itself. A different average mite lifespan according to the season has already been reported by Mikityuk et al. 1976 (Table II). However, it is virtually impossible to test this hypothesis using the living host because, as proven by other studies mentioned above, the health status of the bees changes as well along the season. This issue would be solved only when a standardized artificial feeding system to maintain *V. destructor* without the living host over long periods will be developed. Nowadays, this condition appears much closer than ever before (Piou et al. 2023). Given the importance of an artificial rearing system of *V. destructor* for a wide range of relevant studies, a renewed effort in this direction would therefore be important and highly recommended.

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AUTHORS CONTRIBUTIONS

S.P., F.N. and D.A. conceived the study; S.P., D.F., E.S., V.Z. and, D.A. carried out the survival experiments; S.P. and D.F. analysed the data; S.P. wrote the first draft of the paper; S.P., F.N. and D.A. commented and reviewed the paper.

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DATA AVAILABILITY

The authors declare that the survival data supporting the findings of this study are available upon request.

DECLARATIONS

Competing interests Authors have no competing interests.

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