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RESEARCH

Overall frailty gauged in victims of the Italian plague (Imola, 1630–1632): was plague an indiscriminate killer?

Nicoletta Zedda[1] · Natascia Rinaldo[2] · Emanuela Gualdi-Russo[2] · Barbara Bramanti[1]

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Abstract

Plague is an epidemic-prone infectious disease that has affected humanity with catastrophic effects throughout almost its entire history. One of the most intriguing questions of the last years is whether plague kills indiscriminately. To address the question regarding pre-existent health conditions, this study aims to assess the overall frailty of plague victims and compare it with a sample of non-plague victims from the same period and area. Frailty was assessed using the biological index of frailty (BIF) on two skeletal series dated to the seventeenth century from north-eastern Italy: one of plague victims from the Imola's Lazzaretto ($n=93$) and another from an attritional cemetery located in Ravenna ($n=58$). Comparisons between the BIF values of the two samples were performed separately by sex and age classes. Cox proportional hazards regression was conducted to analyze factors associated with the risk of dying from plague. The age-adjusted ANCOVA test revealed no significant differences in BIF results between the two samples. However, according to Cox's regression, individuals in the lowest BIF category (the least frail) had a significantly higher hazard of dying from plague. Although we found no differences between the mean frailty values of plague and non-plague victims in the univariate analysis, individuals with a low level of frailty showed a higher hazard of dying from plague than from other causes. In fact, otherwise healthier individuals (i.e., with low levels of frailty) could be found only among plague victims.

Keywords Plague · Selective mortality · Sex · Skeletal health · Frailty

Introduction

Plague is an infectious disease caused by the bacterium *Yersinia pestis*. Three main pandemics of plague are recorded in human history: the first plague pandemic spread in Europe and Africa in 541 CE (Little 2007); the second pandemic started in Central Asia in the fourteenth century and rapidly expanded into Europe, where it persisted until the early nineteenth century (Naphy and Spicer, 2004; Bramanti et al. 2016; Spyrou et al. 2022); and finally, the third pandemic spread from China at the end of the nineteenth century (Bramanti et al. 2019, 2016; Xu et al. 2019). Traces of ancient DNA from *Y. pestis* strains (Bos et al. 2011; Bramanti et al. 2021; Guellil et al. 2020, 2021; Haensch et al. 2010; Morozova et al. 2020; Namouchi et al. 2018; Spyrou et al. 2019, 2016, 2022; Susat et al. 2020) confirmed the bacterium as the causative agent of the pandemics.

Genomes of *Y. pestis* have also been reconstructed from human skeletal remains dating back some 5000 years, associated with Mesolithic, Neolithic, and Bronze age cultures (Rasmussen et al. 2015; Valtueña et al. 2017; Spyrou et al. 2018; Rascovan et al. 2019; Susat et al. 2021), while the bacterium is still endemic in some parts of the world, a condition that makes plague one of the infectious diseases that have overwhelmingly influenced human history in the past and still represent a major health burden to date. Evidence of this hazardous condition is the recent plague outbreaks in Uganda, Democratic Republic of Congo, and Madagascar (Demeure et al. 2019; Rubini et al. 2016).

Although *Y. pestis* remains one of the most studied pathogens in the world, its physiopathology is not fully understood.

✉ Natascia Rinaldo
rnlnsc@unife.it

✉ Emanuela Gualdi-Russo
emanuela.gualdi@unife.it

[1] Department of Environmental and Preventive Sciences, Faculty of Medicine, Pharmacy and Prevention, University of Ferrara, Ferrara, Italy

[2] Department of Neuroscience and Rehabilitation, Faculty of Medicine, Pharmacy and Prevention, University of Ferrara, Ferrara, Italy

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Thanks to new high-level technologies, consistent progress has been made in recent years in understanding several aspects of plague, including, but not limited to, pathogenic mechanisms and epidemiology of the pandemics (see e.g., the recent review by Barbieri et al. 2020). Nevertheless, many questions about the past and present of plague remain unanswered. Due to the presence of natural reservoirs of *Y. pestis* in Africa, Asia, and the Americas, and its endemicity in more than 25 countries, it is essential for global health to continue to study the pathogen, understand the illness, and also identify the most vulnerable persons across different societies.

With regard to vulnerability to plague, recent genomics studies, which have identified possible mutations selected to confer resistance or susceptibility to plague, have, however, yielded as yet inconclusive results, mainly due to the objective difficulty of ensuring that *Y. pestis* was indeed the selective agent of the human alleles (Park et al. 2020; Immel et al. 2021). Other research has focused on phenotypic and cultural markers of selection so that, in recent years, archaeoanthropologists, historians, paleoepidemiologists, and biomedical scientists have tried to determine whether plague affected the whole population equally or whether it targeted certain individuals, based on their sex, age, and health conditions.

While most epidemiological studies have reported no significant numerical differences between male and female victims (Alfani and Murphy 2017; Bramanti et al. 2018; Whittles and Didelot 2016), recent research on past plague victims has shown that frailty and age can enhance an individual's risk of dying from plague (DeWitte and Wood 2008; DeWitte 2010a, b; DeWitte 2014; DeWitte and Hughes-Morey 2012; Godde et al. 2020). In contrast, Kacki (2016) argued that in plague pits there were healthier individuals than in attritional cemeteries, concluding that plague did not select its victims based on their pre-existing health. However, the main issue with archeological skeletal series might be the method of assessing pre-existing health, as we can only rely on preserved bones.

To overcome the challenge, several studies have focused on assessing skeletal frailty as a proxy for the overall health status of past populations (Boldsen 2007; DeWitte and Wood 2008; DeWitte 2009, 2012, 2014; Marklein et al. 2016; Marklein and Crews 2017; Godde et al. 2020). Frailty is the physiological stress load that an individual has sustained over a lifetime and that makes him/her more susceptible to disease and death (DeWitte 2010a, b; Zedda et al. 2021). To assess frailty in skeletal remains, bioarchaeologists considered different biomarkers of stress as signals of the entire physiological stress the individuals suffered in life. However, most previous studies on the influence of frailty in determining plague death rate evaluated only a few biomarkers and usually each in an independent survey (DeWitte and Wood 2008; DeWitte 2010a, b; DeWitte 2014; DeWitte and Hughes-Morey 2012; Bramanti et al. 2018; Kacki 2016).

To address the query of whether frailty played a role in plague mortality, we decided to compare two coeval samples, one from an attritional cemetery and one from a plague assemblage from the same geographic region, using, for the first time, a new index, the biological index of frailty (BIF) (Zedda et al. 2021), that considers the coexistence of different biomarkers of frailty on the skeletons, thus provides a more comprehensive overview of frailty during life.

In skeletal assemblages from attritional cemeteries, the frailest individuals of each age group are commonly found, whereas, in catastrophic samples, mortality is apparently far less selective for health characteristics, age, or sex (DeWitte 2010a, b; Kyle et al. 2018; Wood et al. 1992). Considering plague epidemics as catastrophic events, we expect, if plague killed indiscriminately, to find healthier individuals (i.e., with lower frailty) among plague victims than among the victims of an attritional cemetery. Conversely, if plague selected its victims from the frailest individuals, we should find similar incidences of frailty in the plague cemetery as in the attritional ones.

Materials and methods

Materials

We examined two samples dated to the seventeenth century from the Emilia Romagna region (north-eastern Italy). We chose samples from the same historical period and geographic area to minimize any possible differences in population health due to different living and environmental conditions. The first sample consisted of skeletons of plague victims buried during the epidemic of 1630–1632 at the Imola's Lazzaretto. It consisted of 133 skeletons from 4 mass graves (tombs 3, 6, 7, and 8) excavated in 2007 in the complex of L'Osservanza at Imola (BO) (Guellil et al. 2021). The plague epidemic that spread through northern Italy from 1629 to 1632 and killed 25% of the population (Hays 2005) reached the city of Imola in 1630, despite various attempts to stop the spread of the contagion in its course (Cervellati 1986). The monastery L'Osservanza was situated south of the medieval city walls and was used as a "Lazzaretto," a place of care for infected people from both the city and the countryside, as well as a burial place for the dead (Cervellati 1986). Historical sources explicitly described the symptoms of affected individuals and mentioned "buboes" among other pathognomic signs (Cervellati 1986). The archeological and historical evidence suggests that the individuals in the mass graves were the victims of a major epidemic event (Guellil et al. 2021). Genomic analyses performed on 15 individuals

from the mass graves yielded ancient *Y. pestis* DNA from three individuals (Guellil et al. 2021), further confirming that plague was the cause of the catastrophic burials in the lazaretto.

The second sample consisted of non-plague victims from the lower level of the attritional cemetery of the church of San Biagio in Ravenna. The church cemetery in the village of San Biagio, placed on the border of Ravenna, was used from 1602 to 1817, as attested in the parish register of the Archdiocese of San Biagio (Caravita 2008). Historical records confirmed that it was not used for plague victims. Archeological excavations, which began in 2013, recovered two batches of burials dating from the period between 1600 and the early 1800s that contained over 200 inhumations. According to the stratigraphic data, the lowest burials most likely dated from the seventeenth century and are the ones used in our analysis.

Following the guidelines of Zedda et al. (2021), we analyzed all individuals over the age of 11, for a total of 93 individuals from Imola and 58 from Ravenna. All skeletons were stored and investigated at the Laboratory of Archaeo-Anthropology and Forensic Anthropology, University of Ferrara, Italy.

Table 1 Comparison between biomarker frequencies of the two samples (plague victims and non-plague victims). Relative frequencies (in percentage) were calculated on the total number of individuals on which the biomarker was detectable

Biomarkers	Plague's victims (Imola) *N* (%)	Non-plague's victims (Ravenna) *N* (%)	*p*
Short stature	11 (25%)	13 (28%)	0.7736
Low body mass	14 (37%)	10 (26%)	0.3236
Linear enamel hypoplasia	37 (97%)	20 (91%)	0.3015
Cribra orbitalia	13 (32%)	8 (32%)	0.9802
Porotic hyperostosis	38 (83%)	21 (70%)	0.1973
Rickets	2 (4%)	1 (2%)	0.6685
Periodontitis	28 (65%)	24 (80%)	0.1669
Periostitis	9 (18%)	16 (39%)	0.0254*
Joint disease	13 (82%)	44 (98%)	0.0518(*)
Vertebral disease	11 (58%)	38 (86%)	0.0126*
Trauma	7 (28%)	7 (21%)	0.5084
Osteoporosis	6 (54%)	0 (0%)	-

*significant *p* value

(*) almost significant *p* value

Methods

The skeletal remains were carefully cleaned and restored; age-at-death estimation and sex diagnosis were assessed for each individual from San Biagio (Ravenna). Sex diagnosis and age-at-death estimation for the Imola sample had been performed previously (Guellil et al. 2021; Rinaldo et al. 2014; Rubini et al. 2016).

We assessed sex in adult individuals and adolescents older than 15 years using morphological methods relying on sexual dimorphic features of the skull and pelvis (Acsadi and Nemeskeri 1974), when applicable. Alternatively, and only for adults, metric methods (Bass 1995; France 1998; Gualdi-Russo 2007; Manolis et al. 2009; Scheuer and Elkington 1993) were applied to the post-cranial skeleton. To estimate age-at-death, we used several published methods based on the development (for adolescents) and aging (for adults) of different parts of the skeleton (Belcastro et al. 2008; Brothwell 1981; Cardoso and Severino 2010; Işcan and Kennedy 1989; Buikstra and Ubelaker, 1994; Lovejoy 1985; Meindl and Lovejoy 1985; Ríos and Cardoso 2009; Scheuer and Black 2004; Suchey et al. 1986; Todd 1920; Ubelaker 1989). Individuals were then grouped into age classes according to the classification proposed by Buikstra and Ubelaker (1994).

To gauge frailty, we applied the recently published BIF (Zedda et al. 2021). Firstly, we checked for the presence or absence of 12 biomarkers of physiological stress, following the guidelines outlined by Zedda et al. (2021). The list of biomarkers can be found in Table 1. At least three of the biomarkers proposed by Zedda et al. (2021) should be observable on the skeleton to apply the BIF. Each biomarker correlates with non-specific stress and is associated with an increased risk of death. Therefore, the BIF, more than any single biomarker, offers a comprehensive view of the frailty of each individual and can be used as a proxy for his or her pre-existing health status and, thus, for the risk of death. Stature was estimated from long bone measurements (Gualdi-Russo et al. 2018; Pearson 1899; Trotter and Gleser 1958), whereas osteoporosis was evaluated through quantitative ultrasonometry (Rinaldo et al. 2018). Each biomarker was considered as a categorical variable, assigning a score of 1 if present and 0 if absent.

For each individual, the index was calculated from the weighted mean of all observable biomarkers (the weight of each biomarker was calculated by Zedda et al. (2021)). The formula for calculating the index is

$$BIF = \frac{\Sigma(Weight * Score)}{\Sigma(Weight)} * 100$$

The resulting BIF values can be divided into three categories of frailty: low (values between 0 and 21), medium (values between 21 and 53), and high (values between 53 and 100) (Zedda et al.et al. 2021).

Statistical analysis

Descriptive statistics were performed by calculating means and SDs for continuous variables (including BIF) and frequencies for categorical variables.

Differences between the BIF values of plague and non-plague victims, between sexes, and across age classes were assessed using the *t*-test, the Mann–Whitney *U* test, and the analysis of covariance (ANCOVA) adjusted for age, according to the distribution of the data. A comparison between BIF categories was performed with the chi-square test. We applied chi-square tests also on the frequencies obtained for each biomarker to identify any statistically significant differences between plague and non-plague victims and between sexes.

Cox proportional hazards regression was carried out pooling the two samples to determine the impact of their frailty measures on the risk of death from plague. As a binary outcome, we selected "death from plague" (event of interest, coded as 1 and assumed for individuals from Imola) or "death by other causes" ("attritional" deaths, coded as 0 and assumed for individuals from Ravenna). As survival time, we entered the age-at-death of the individuals, i.e., the mean age in the death interval assessed for each individual by anthropological methods, and as predictor variables sex (dichotomous variable) and BIF categories.

Each p-value < 0.05 was considered statistically significant.

All statistical analyses were conducted using STATISTICA (version 11, StatSoft, Tulsa, OK, USA) and the Statistical Software MedCalc version 14.8.1 (MedCalc Software bvba, Ostend, Belgium).

Results

Frailty assessment

The index of frailty BIF was calculated for all individuals older than 11 years at the time of death, for whom at least three biomarkers of stress were observable. The estimation of frailty through the BIF was possible on 65 plague victims and 52 non-plague victims (Table 2).

As reported in Table 2, we could detect no statistically significant differences in the mean frailty values of the two samples. However, we noted that the total mean BIF value, regardless of the sex of the victims, was higher in the Ravenna sample, indicating a higher degree of frailty, as would be expected in an attritional cemetery (Fig. 1). This observation is corroborated by the comparison of the BIF categories, which showed a significantly higher percentage of high frailty individuals in the Ravenna sample and none in the lowest frailty category (Table 2).

Table 2 Comparisons between mean values of the biological index of frailty (BIF) of the two samples (plague victims vs. non-plague victims). The total value also includes individuals for whom sex and/or adult age category could not be determined

	Plague victims (Imola)		Non-plague victims (Ravenna)		*p*
	N	Mean *BIF* (*SD*)	*N*	Mean *BIF* (*SD*)	
Total	65	40.21 (24.46)	52	46.10 (21.11)	0.1724[a]
Males	30	42.81 (21.48)	27	42.52 (18.62)	0.9572[a]
Females	35	37.99 (28.86)	22	48.40 (20.83)	0.1277[a]
12–17	13	31.23 (18.19)	2	58.82 (58.23)	0.4969[b]
18–34	32	47.26 (24.78)	10	43.69 (20.48)	0.6469[b]
35–50	14	38.06 (25.87)	22	46.94 (19.43)	0.4550[b]
50+	5	32.58 (22.13)	14	46.29 (17.43)	0.3085[b]
BIF categories		*N* (%)		*N* (%)	0.0333[c]
Low		8 (13.6)		0 (0.0)	
Medium		30 (50.8)		26 (56.5)	
High		21 (35.6)		20 (43.5)	

[a]Comparisons performed using ANCOVA adjusted for age

[b]Comparisons performed using *U* Mann–Whitney statistical test

[c]Comparisons performed using chi-square statistical test

When comparing males and females separately, we observed almost no difference between the males of the two groups, who showed very similar values of frailty (Table 2). Quite different was the situation of the females, who showed greater dissimilarity, although not statistically significant, with lower frailty values in Imola's sample, indicative of the generally better health status of the female plague victims (Fig. 1). Nevertheless, the comparison of the BIF values within each population did not reveal any significant difference between males and females (Imola $p = 0.4324$; Ravenna $p = 0.3030$).

Regarding dissimilarities between individuals of different age classes (Table 2), the mortality rates for Imola show the typical pattern of other plague sites, i.e., a lower peak of mortality during late childhood and a second more prominent peak for young adults (18–34) (Bramanti et al. 2018; Guellil et al. 2021; Rubini et al. 2016). The distribution is very different in the attritional cemetery of Ravenna, where mortality clearly increases with age, particularly after the age of 35 (Table 2). The difference in mortality rates for different age groups across the two samples is statistically significant ($p < 0.0001$).

We compared the BIF values of each age class with the Mann–Whitney *U* test (Table 1); although there are some obvious differences, none are statistically significant. We noted a high mean BIF value in Ravenna adolescents, yet, with a high standard deviation, due to the low number of individuals ($N = 2$) in this class. We also noted that, although not statistically significant, the mean BIF value was always

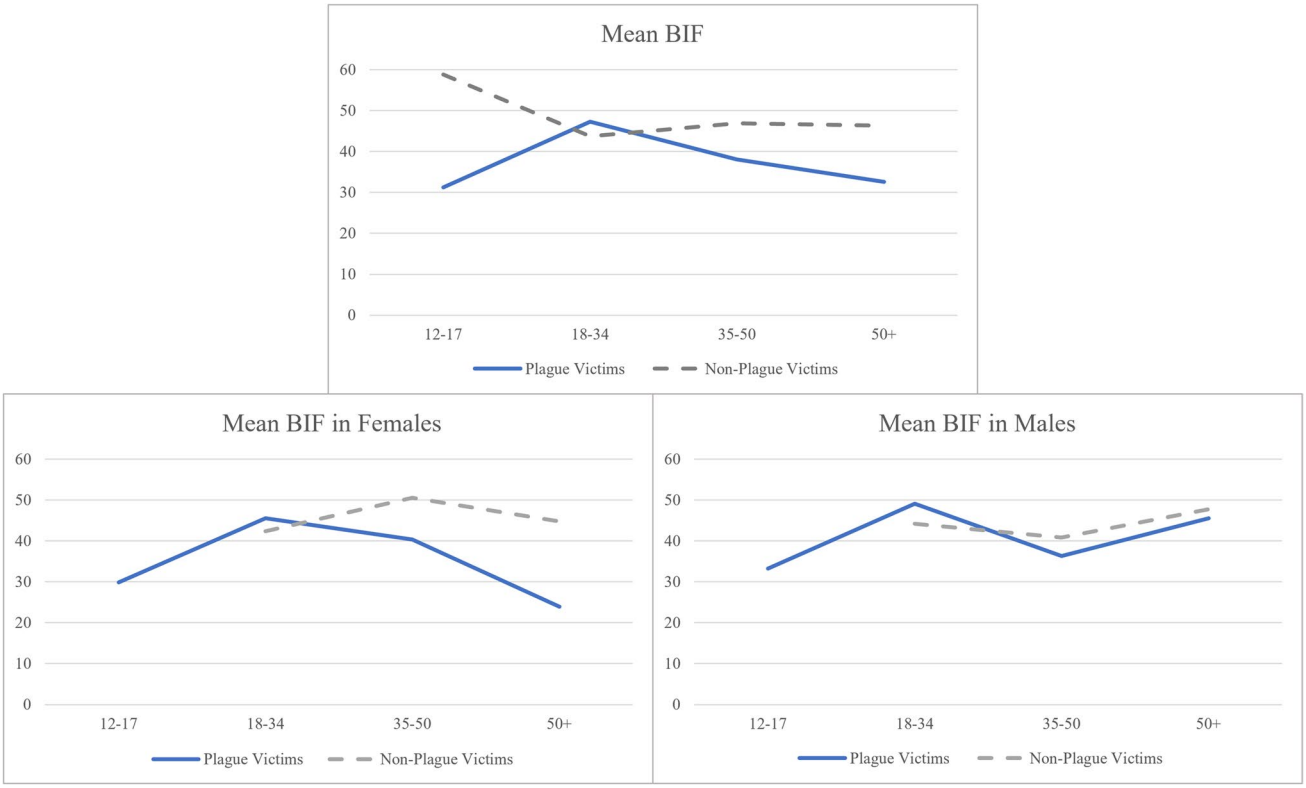

Fig. 1 Graphs of mean BIF values in plague and non-plague victims for the different age classes and sexes

higher in non-plague victims with the only exception of the 18–34-year-old group. ANOVA calculations among the different age classes within each site were also not significant (Ravenna $p = 0.8267$; Imola $p = 0.1637$).

As far as the gross frequencies of each biomarker are concerned, we observed (Table 1) some significant differences between the two groups: all the biomarkers linked to advanced age (periodontitis, joint diseases, vertebral diseases), as well as periostitis, displayed higher frequencies in the Ravenna assemblage, apart from osteoporosis, for which the only cases were recovered in the Imola sample. The only statistically significant differences concerned vertebral diseases, periostitis, and osteoporosis, while for joint diseases, the difference was very close to the significance threshold (Table 1).

Analyzing in more detail the frequencies of cribra orbitalia (CO) and porotic hyperostosis (PH) (Table 3), we noted that there were no statistically significant differences between Imola and Ravenna, both as regards the presence and absence, as well as the degrees of severity and healing of the lesions. Males and females of both groups did not show any distinct pattern, even in this aspect. We observed, however, that individuals showing healed CO (degree 3–4) were all plague victims (both males and females) (Table 3), a situation that suggests, at least as far as CO is concerned, that most plague victims had overcome a state of iron deficiency.

Table 3 Comparison between CO and PH frequencies of the two samples (plague victims and non-plague victims). Relative frequencies (in percentage) were calculated on the total number of individuals that manifested porous lesions

	Plague's victims (Imola) *N* (%)	Non-plague's victims (Ravenna) *N* (%)	*p*
Cribra Orbitalia			
Males	4 (33%)	3 (43%)	0.8232
Females	8 (67%)	4 (57%)	0.6098
Healing			
Active (degree 1)	2 (15%)	3 (37.5%)	0.6098
Healing < 50% (degree 2)	6 (46%)	5 (62.5%)	
Healing > 50% (degrees 3–4)	5 (39%)	0 (0%)	
Porotic hyperostosis			
Males	18 (47%)	11 (58%)	0.6473
Females	19 (53%)	8 (42%)	0.0782
Healing			
Active (degree 1)	0 (0%)	0 (0%)	0.0782
Healing < 50% (degree 2)	1 (3%)	1 (5%)	
Healing > 50% (degrees 3–4)	37 (97%)	19 (95%)	

Survivorship

To further investigate how differences in frailty influenced the mortality risk in the two samples, we applied a Cox hazard regression model to the individuals according to their frailty and sex. The results of the Cox proportional hazard regression are shown in Table 4: the hazard ratio (HR) was not significant between the two sexes, indicating that males and females had relatively the same risk of dying from plague. However, when sex is held constant, individuals with low frailty had an almost three times higher hazard of dying from plague than individuals with medium or high frailty.

Discussion and conclusions

In this study, we set out to understand whether plague victims were selected based on their health status, as some scholars suggested (DeWitte and Wood 2008; DeWitte and Hughes-Morey 2012; DeWitte 2018), or not, as argued by others (Bramanti et al. 2018; Kacki 2016).

Despite this topic was highly debated in the last years, studies on the effect of frailty on plague mortality, which compared plague and non-plague cemeteries, gave inconsistent and contradictory results, and this might be due to the fact that the biomarkers used as a proxy for frailty were different and were mostly used independently. Therefore, we proposed here the first study on plague victims using a newly developed frailty index, the BIF, which allows an overall assessment of frailty through the investigation of up to 12 markers of biological stress (Zedda et al. 2021).

The skeletal sample from the Imola lazaretto was compared with skeletons from a regular cemetery in the same geographic region and the same historical period. During the seventeenth century, the region was affected by various calamities, from famines to epidemics, and by the civil wars and economic instability that characterized the entire period of the Little Ice Age, particularly the sixteenth and seventeenth centuries (Baldini and Bedeschi 2018). Belonging to the same geographical area and historical timeframe means that environmental, nutritional, and climatic conditions, as well as all social and historical circumstances that can influence frailty, are similar between the two groups, making plague the main discriminating variable.

The rationale for this comparison is that if plague did not select its victims from the most fragile individuals, we would expect to find more signs of stress, and thus a higher index of frailty, in the skeletal series of the Ravenna attritional cemetery. If conversely plague selected its victims according to frailty, we should have found similar BIF values in the two cemeteries. In fact, it is generally accepted that skeletal assemblages from attritional cemeteries are represented by individuals of all age groups who were more fragile than the surviving population (Brzobohatáet al. 2019; DeWitte and Stojanowski 2015; DeWitte and Wood 2008; Kyle et al. 2018; Margerison and Knüsel 2002).

From the comparison of the two mean values of BIF with the ANCOVA analysis, we could not appreciate any significant difference between plague and non-plague victims, a condition that might suggest that plague selected its victims from the frailer group. However, it must be considered that average values may flatten the results, reducing the potential of the BIF index to identify classes or groups with lower or higher frailty. As a matter of fact, observing the mean BIFs of the two populations, we noted that the people buried in the Imola lazaretto were less frail, especially the women. Except for the 18–34 age class, all other age classes also appeared to be less frail in plague than in non-plague individuals. Indeed, when we compared the frailty categories of the two samples, regardless of age and sex, we found a statistically significant difference, as low frailty individuals (i.e., healthier) were only among the plague victims. The absence of individuals with low frailty is to be expected when analyzing an attritional cemetery, but the presence of individuals with low frailty in the plague sample emphasizes that plague did not select only frail individuals.

This finding is also supported by the Cox proportional hazards model, which shows that individuals with a low

Table 4 Results of Cox proportional hazard regression

Covariates	*B*	*SE B*	*p*-value	*HR*	95% CI *HR*
Sex					
Male (reference)	-	-	-	-	
Female	0.1510	0.2776	0.5864	1.1630	0.6769 to 1.9984
BIF					
Low frailty (reference)	-	-	-	-	
Medium frailty	− 1.0622	0.4240	0.0122	0.3457	0.1512 to 0.7903
High frailty	− 1.0223	0.4323	0.0180	0.3598	0.1548 to 0.8359

B coefficient that determines the impact of the covariates, *SE* standard error of B, *HR* hazard ratio, *CI* confidence interval. An HR < 1 indicates a reduction in the hazard, meanwhile, an HR > 1 indicates an increase in the hazard

level of frailty had a three times higher hazard of dying from plague in comparison to individuals with medium or high frailty levels, who had the same hazard. The same model also shows that males and females had the same hazard of dying from plague.

Godde et al. (2020) ran the same statistical model on London victims of the Black Death and other English non-plague victims, with the result that individuals with high frailty had a higher risk of dying from plague. However, Godde et al. used only the presence of one of the indicators (LEH, CO, or short stature) as a proxy for frailty and could therefore not distinguish between different levels of expression (i.e., of frailty).

DeWitte (2010a, b), again analyzing the victims of the plague in London, found similar results but noted fewer markers of stress in female plague victims and concluded that plague killed more otherwise healthy women than healthy men, an observation apparently similar to ours. However, in DeWitte's study, the individuals from London were not compared to non-plague victims from the same geographical area and historical period. Furthermore, we would like to emphasize once again that the index of frailty BIF allows for a more comprehensive evaluation of an individual's frailty than single biomarkers of stress.

In fact, the comparison of the single biomarkers in our study reveals few significant differences; in particular, joint and vertebral diseases, as well as periostitis, are present with a higher frequency in non-plague victims. Since all these biomarkers are usually more represented in older adults, it is not surprising that they were scored more frequently in the individuals of the attritional cemetery. We indeed know that a low number of deaths in adolescence and an increasing mortality rate throughout adulthood are consistent with attritional mortality (Brzobohatá et al. 2019), and we see this phenomenon in the Ravenna sample (Table 2), while in plague cemeteries, we have a very characteristic mortality curve (Bramanti et al. 2018) with a higher percentage of young adults than older adults, as in the case of Imola (Rubini et al. 2016; Guellil et al. 2020). In contrast, we observed that osteoporosis, despite being a biomarker mostly associated with older age, was more present in plague victims, although the sample is too small to draw any conclusions.

We looked more specifically at CO and PH, with a focus on the healing of the lesions. While the exact etiology of these two pathologies is still much debated, different studies on iron deficiency anemia, both clinical (Kent et al. 1994) and epidemiological (Cook 1990), support the hypothesis of a causal link between these cranial lesions and anemia (Mcilvaine 2015; Rivera and Mirazón Lahr 2017; Brickley 2018; Godde and Hens 2021). Several studies on *Y. pestis* have highlighted how iron is crucial for the reproduction cycle of the bacterium and how *Y. pestis*' growth is in some way inhibited by low availability of free iron and zinc in the body of the host (Zauberman et al. 2017; Demeure et al. 2019). It has previously been proposed that iron-deficiency anemia could represent a protective factor against plague infection (Ell 1984, 1985), but to our knowledge, this theory has never been investigated in skeletal assemblages of plague victims. We found no evident statistical difference between the two populations, even when we considered the severity and healing status of the lesions, suggesting that iron deficiency was likely not protective against plague. However, it is worth mentioning that also other forms of anemias could have caused the lesions, as well as nutritional deficiencies, iron deficiency caused by parasites (Godde and Hens 2021), and other chronic diseases, or inflammatory conditions (Camaschella 2015; Godde and Hens 2021). Regardless, it was among plague victims that we found the higher number of individuals with healed lesions, which is again indicative of a healthier status of the plague victims. However, individuals with such health conditions should have been more susceptible to infections, and therefore, we should have observed a greater presence of active lesions in plague victims.

In conclusion, in this study, we demonstrated that although also frail individuals died from plague at Imola (in particular adults aged 18–34 years), those with a lower level of frailty (i.e., healthier) had a higher hazard of dying from plague than from other causes.

Although, in comparison to other indices of frailty, the BIF allows for a larger number of individuals to be analyzed as only at least three of the proposed biomarkers should be observable on the skeleton (Zedda et al. 2021), a limitation of this study remains the low sample size. Thus, it would be useful to investigate other plague contexts using BIF to observe whether the trend we saw here is a rule or merely a local effect.

Author contribution N.Z. and N.R. wrote the original draft. B.B. and E.G.-R. critically reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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