Suicide and All-Cause Mortality Within 1 Year After a Suicide Attempt in the VigilanS Cohort

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Abstract

Objective: Obtaining better knowledge on the outcomes of patients who attempt suicide is crucial for suicide prevention. The aim of our study was to determine the causes of death 1 year after a suicide attempt (SA) in the VigilanS program, mortality rates, and risk factors associated with any cause of death and suicide.

Methods: A prospective cohort of 7,406 people who had attempted suicide between January 1, 2017, and December 31, 2018, was included in the study. The vital status of each participant was sought, and the cause of death was established through a phone call to their general practitioner or psychiatrist. Second, the relationship between sociodemographic and clinical factors and death by suicide within 1 year of an SA was assessed using a multivariable Cox model.

Results: At 1 year, 125 (1.7%) participants had died, 77 of whom died by suicide. Half of the deaths occurred within the first 4 months after an SA. Hanging (20.3%; 24/125) and self-poisoning (19.5%; 23/125) were the methods the most often used for suicide. We demonstrated that male sex (HR = 1.79 [1.13–2.82], $P = .01$) and being 45 years of age or older (between 45 and 64 years old, HR = 2.08 [1.21–3.56], $P < .01$; 65 years or older, HR = 5.36 [2.72–10.54], $P < .01$) were associated with a higher risk of death by suicide 1 year after an SA and that being younger than 25 years was associated with a lower risk (HR = 0.22 [0.07–0.76], $P = .02$).

Conclusions: One out of 100 people who attempted suicide died by suicide within 1 year after an SA. Greater vigilance is required in the first months following an SA, especially for males older than 45 years.

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Suicide is a leading cause of avoidable death worldwide, with approximately 700,000 deaths per year.[1] Moreover, with at least 16 million per year, suicide attempts (SAs) are estimated to be 25 to 50 times more common than deaths by suicide. However, even if scientific literature has investigated the relationship between sociodemographic and clinical factors and the risk of death by suicide, the trajectory of people who have attempted suicide remains unclear.[2]

In this context, most studies have focused on the rate of death by suicide, as suicide is one of the preventable and well-known causes of death after an SA. Therefore, a recent meta-analysis estimated high rates of death by suicide after an SA (ie, 2.8% at 1 year, 5.6% at 5 years, and 7.4% at 10 years).[3] Nonetheless, while most deaths after an attempt are probably due to natural causes and not to suicide, few studies have precisely examined the cause-specific distribution of post-SA mortality.[4,5] These few studies have divided the causes into natural, external (suicide, accidents, homicides), and uncertain causes.[6,7] In addition, most studies have included a relatively high number of undetermined causes of death (eg, 5% in a Swedish cohort study, 17.5% in a French register-based study, up to 36% in a Canadian cohort study).[4,8–12] In summary, the current data and literature lead to uncertainties and imprecision regarding the outcomes of patients after an SA.

The literature is more accurate regarding the outcomes of patients who engage in deliberate self-harm (DSH), which includes self-injuries without suicidal intent and SAs.[13,14] After DSH, most natural deaths seem to be due to diseases of the circulatory system, digestive system, and neoplasms.[15] However,

Clinical Points

- What becomes of patients who have attempted suicide is unclear. In this prospective cohort of 7,406 people who had attempted suicide, 125 (1.7%) had died within 1 year after the attempt, 77 of whom had committed suicide.
- Male sex and being older than 45 years were associated with a higher risk of death by suicide, and being younger than 25 years was associated with a lower risk.
- Greater clinician vigilance is required following a suicide attempt, especially for male patients older than 45 years.

the epidemiology, rates, and causes of death after any SA and DSH are probably not similar. Obtaining better knowledge on what becomes of suicidal patients, and therefore determining the most at-risk periods and associated risk factors, is crucial for suicide prevention.

Every year, approximately 10,000 people die by suicide in France.[16] While the rates of suicide have been declining in recent decades, it remains a major public health issue in our country.[17] Recently, the VigilanS suicide prevention program was created to provide a brief contact intervention and to follow-up patients after hospital discharge for an SA.[18] Patients are included in the program regardless of their psychiatric diagnosis, age, or inclusion location.

Aims of the Study

The aim of our study was to determine the causes of death 1 year after an SA, mortality rates, and risk factors associated with any cause of death and suicide using a cohort from the VigilanS program.

METHODS

Study Design

A prospective cohort of 7,406 subjects was included in the study between January 1, 2017, and December 31, 2018. All subjects admitted to health care services in the Hauts-de-France region for an SA were included in the VigilanS brief contact intervention program.

VigilanS is a brief contact intervention combining resource cards, telephone calls, and mailings for patients with an SA.[18] All attempters received a resource card at inclusion. Those with a history of a prior SA received a phone call within 10 days after the attempt. Finally, all attempters received a phone call at 6 months after the SA. The intervention was conducted by a team of mental health care professionals specially trained in suicidal crisis management from the University Hospital of Lille. The included patients were men and women, regardless of age, who had survived an SA. The suicidal intent of the act was evaluated by a psychiatrist before inclusion during an interview. A suicide attempt was considered as "a situation in which a person has performed an actually or seemingly life-threatening behavior with the intent of jeopardizing his life, or to give the appearance of such an intent, but which has not resulted in death."[19] Subjects were excluded from the study if they refused to participate or if they died during the index hospital stay.

The VigilanS study was authorized by the French Ministry of Health and approved by the Ethics Committee of the Nord-Pas-de-Calais region, the Commission Nationale Informatique et Liberté (CNIL), and the Local Data Protection Service. In accordance with this legal status, professionals ensure the patient's compliance after complete oral and written information is given, and no signed informed consent is required. The study was registered with ClinicalTrials.gov (NCT03134885).

Data Collection

At inclusion, data on sociodemographic characteristics (age, sex), the method of the SA (poisoning, cutting or piercing, hanging, drowning, jumping from a height, use of a firearm), whether the SA was associated with acute alcohol use or medication overdose, and the history of prior SAs were collected for each patient. The inclusion location (intensive care unit [ICU], general hospital wards, or other locations [emergency department, psychiatric department, pediatrics department]) was also recorded to assess the severity of the SA. The SA was considered serious if the patient was hospitalized in the ICU or general hospital wards following the attempt. The name of their general practitioner or psychiatrist was collected.

From March 2019 to January 2020, the vital status of all patients at 1 year was sought through letters sent to the town halls of birth and residence and to the patients' general practitioners (Supplementary Figure 1). Since January 2020, the online availability of the French national mortality database by the French National Institute for Statistical and Economic Studies (INSEE) has permitted us to check the 1-year vital status of all patients in the cohort and to determine the eventual dates of death.

Finally, for the patients who died within 1 year after inclusion, the cause of death was first established through a phone call to their general practitioner or psychiatrist, as a large number of causes of death are unspecified in the French mortality database.[8] When the cause of death remained undetermined, the national French death register "CépiDc" was consulted to find the last cause of death.

Primary and Secondary Outcomes

The primary outcome was death by suicide within 1 year after inclusion. The secondary outcome was any cause of death within 1 year.

Statistical Analysis

Descriptive analysis. Descriptive statistics were calculated for the variables of interest. Continuous variables are presented as the means and standard

deviations (SDs). Discrete variables are expressed as frequencies and percentages. For patients whose vital status was known, Kaplan-Meier curves were used to represent time from the index SA to death by any cause or suicide. For those who died by suicide, survival curves were also stratified according to sex and age group of the participants.

Analysis of the primary outcome. First, the rate of death by suicide at 1 year was computed. Then, the age-standardized incidence rate of deaths by suicide per 100,000 person-years (PYs) based on the European population was calculated.[20] Second, the relationship between sociodemographic factors (age, sex), clinical factors (the inclusion location, method used for the SA, duration of hospitalization), and death by suicide within 1 year was assessed using a bivariate Cox model. Other causes of death were considered censored. Then, variables with a *P* value < .2 in the previous analysis were included in a multivariable Cox model, and *P* values < .05 were considered significant.[21,22] The factors associated with all-cause mortality were considered as the secondary outcomes and evaluated with the same method. R software version 3.6.1 was used for all analyses.

Sensitivity analysis. The relationship between sociodemographic and clinical factors and death by suicide within 1 year was assessed using a bivariate and then multivariable Cox model after removing the subjects with an unknown cause of death. Then, the association between the first phone call within 10 days and death by suicide was assessed with the same method (bivariate and multivariable Cox model).

Table 1.
Baseline Characteristics of the Participants[a]

Characteristics	All patients (n = 7,406)	All deaths (n = 125)	Deaths by suicide (n = 77)
Age			
Mean (± SD), y	38.3 (± 16.4)	55.2 (± 17.0)	51.8 (± 16.5)
< 25 y	2,004 (27.1)	3 (2.4)	3 (3.9)
25–44 y	2,544 (34.4)	26 (20.8)	20 (26.0)
45–64 y	2,440 (32.9)	61 (48.8)	39 (50.6)
≥ 65 y	418 (5.6)	35 (28.0)	15 (19.5)
Women	4,548 (61.4)	58 (46.4)	35 (45.5)
Men	2,858 (38.6)	67 (53.6)	42 (54.5)
First attempt	3,952 (53.4)	63 (50.4)	39 (50.6)
Inclusion location			
Other departments[b]	5,279 (71.4)	38 (30.4)	18 (23.4)
General hospital wards	1,867 (25.3)	5 (4.0)	5 (6.5)
Intensive care unit	242 (3.3)	82 (65.6)	54 (70.1)
Method of suicide attempt			
Poisoning	5,840 (78.9)	92 (73.6)	57 (74.0)
Cutting or piercing	615 (8.3)	12 (9.6)	7 (9.1)
Hanging	388 (5.2)	5 (4.0)	4 (5.2)
Jumping from a height	130 (1.8)	4 (3.2)	2 (2.6)
Drowning	50 (0.7)	2 (1.6)	1 (1.3)
Use of a firearm	34 (0.5)	0	0
Other	349 (4.7)	10 (8.0)	6 (7.8)
Suicide attempt with drug overdose	6,026 (81.4)	95 (76.0)	60 (77.9)
Suicide attempt with acute alcohol use	3,493 (47.1)	68 (54.4)	45 (58.4)

[a]Values expressed as n (%) unless otherwise noted.
[b]Emergency departments, psychiatric departments, pediatric departments.

RESULTS

Patients at Inclusion

A total of 7,406 patients were included in our study and followed up for 1 year (Table 1). A small number of patients (58 patients) were excluded from the study (ie, refused to participate or died during the index hospital stay). The mean age of the subjects was 38.3 (± 16.4) years. More than half of the subjects were first-time attempters (53.4%) and women (61.4%). Most of the patients (71.4%) were admitted to emergency departments, psychiatric departments, or pediatric departments after their SA. A few of them were admitted to intensive care units (3.3%), and a quarter were admitted to general hospital wards (25.3%). Self-poisoning was the most often used SA method (78.9%), and half of the SAs were associated with acute alcohol use (47.1%).

Causes of Death Within 1 Year

One year after an SA, of 7,406 patients, 125 (1.7%) died, 77 (1.0%) of whom had committed suicide (see Figure 1). Four causes of death (3.2%) remained unknown at the end of the study. Suicide was the most frequent cause of death in our cohort (77 of 125 patients [61.6%]), followed by circulatory diseases (19.5%) (see Supplementary Table 1). Among those who died by suicide, nearly a third (n = 23) used poisoning, and another third (n = 24) used hanging as the suicide method. In about another third of the patients (n = 23), the method used for suicide remained unknown. The mean age of those who died by suicide was 51.8 (± 16.5) years. Half of them were between 45 and 64 years old (50.6%) and were first-time attempters (50.6%) at inclusion.

While the crude incidence rate of death by suicide is 1,039 per 100,000 PYs in our study, the age-standardized suicide rate (based on the European population) was estimated at 1,415 per 100,000 PYs.

Survival Curve Analysis

Among those who died within 1 year, the survival curves estimated according to any cause of death or suicide are presented in Figure 2. Within 1 year after an SA, half of the deaths from any cause and from suicide occurred within the first 4 months. Moreover, the all-cause and suicide mortality curves evolved in parallel.

Almost all people who attempted suicide at less than 25 years old had a high survival probability within 1 year for women and men (see Figure 3). In contrast, men aged over 64 years had the lowest probability of survival at 1 year (95%).

Figure 1.

(A) Cause of Death Within 1 Year After a Suicide Attempt (N = 125) and (B) the Suicide Method Used

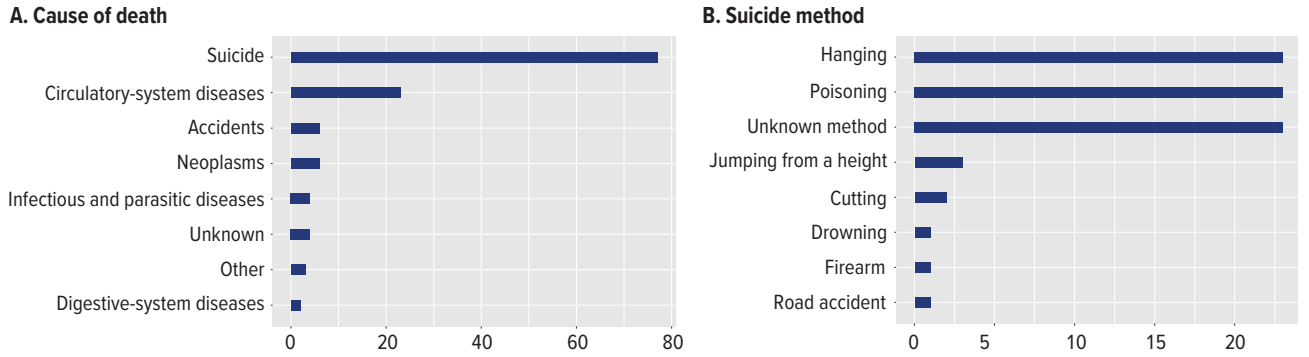
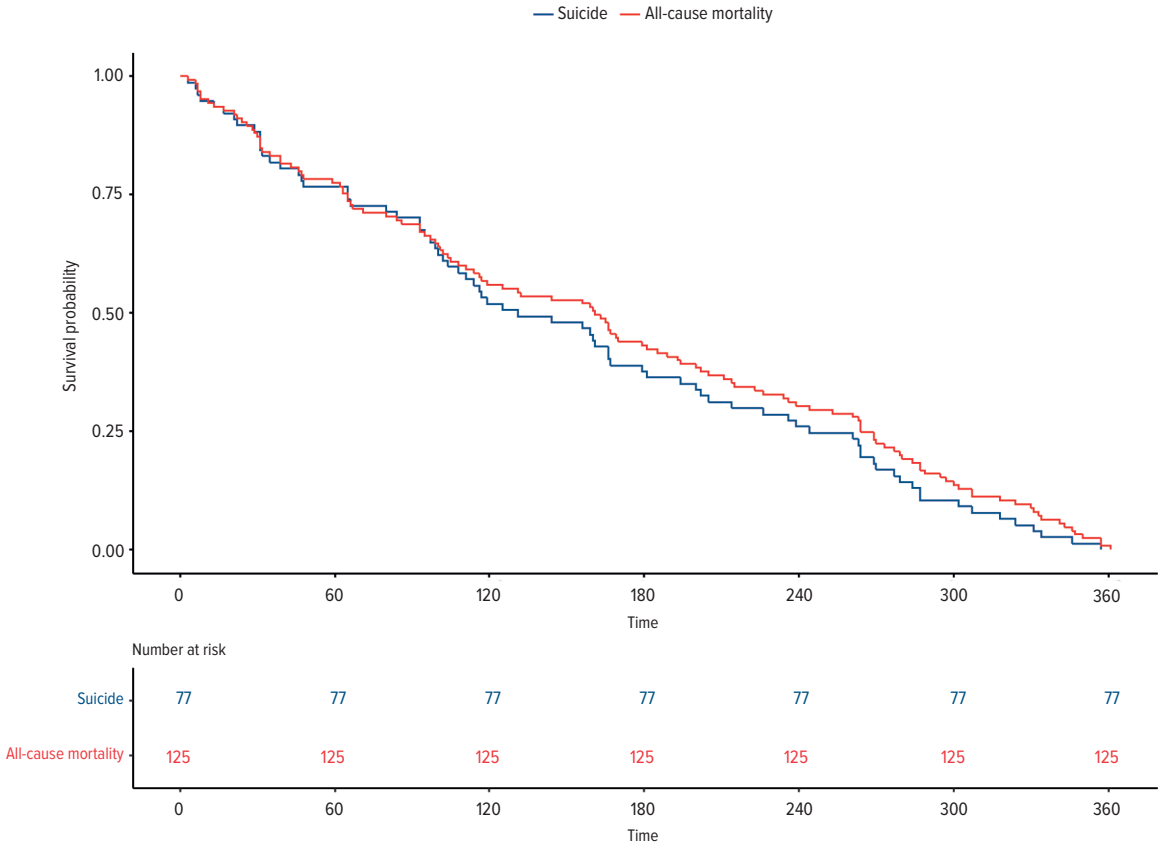


Figure 2.

Kaplan-Meier Curves of All-Cause and Suicide Mortality Within 1 Year

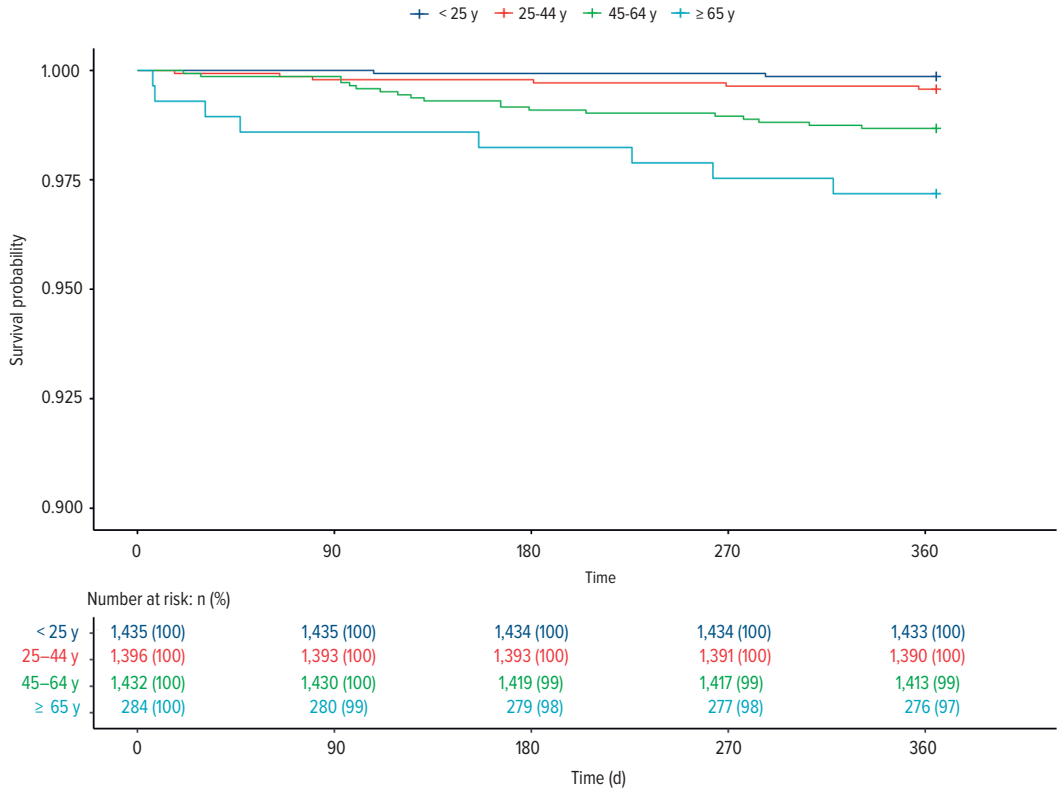


Factors Associated With the Risk of Death by Suicide Within 1 Year

According to the multivariable Cox model, being less than 25 years old was statistically associated with a lower risk of death by suicide within 1 year (HR = 0.22 [0.07–0.76], $P = .02$) compared to being aged 25 to 44 years (see Table 2). Being between 45 and 64 years old (HR = 2.08 [1.21–3.56], $P < .01$) and being older than 64 years of age (HR = 5.36 [2.72–10.54], $P < .01$), compared to being 25 to 44 years old, were associated with a higher risk of future death by suicide. Male sex was also statistically associated with death by suicide within 1 year (HR = 1.79 [1.13–2.82], $P = .01$). The inclusion location and acute alcohol use during the last SA were not statistically associated with death by suicide within 1 year after adjustment for confounding variables ($P > .05$).

Figure 3.
Death by Suicide Within 1 Year in (A) Women and (B) Men According to Age Group

A. Women



B. Men

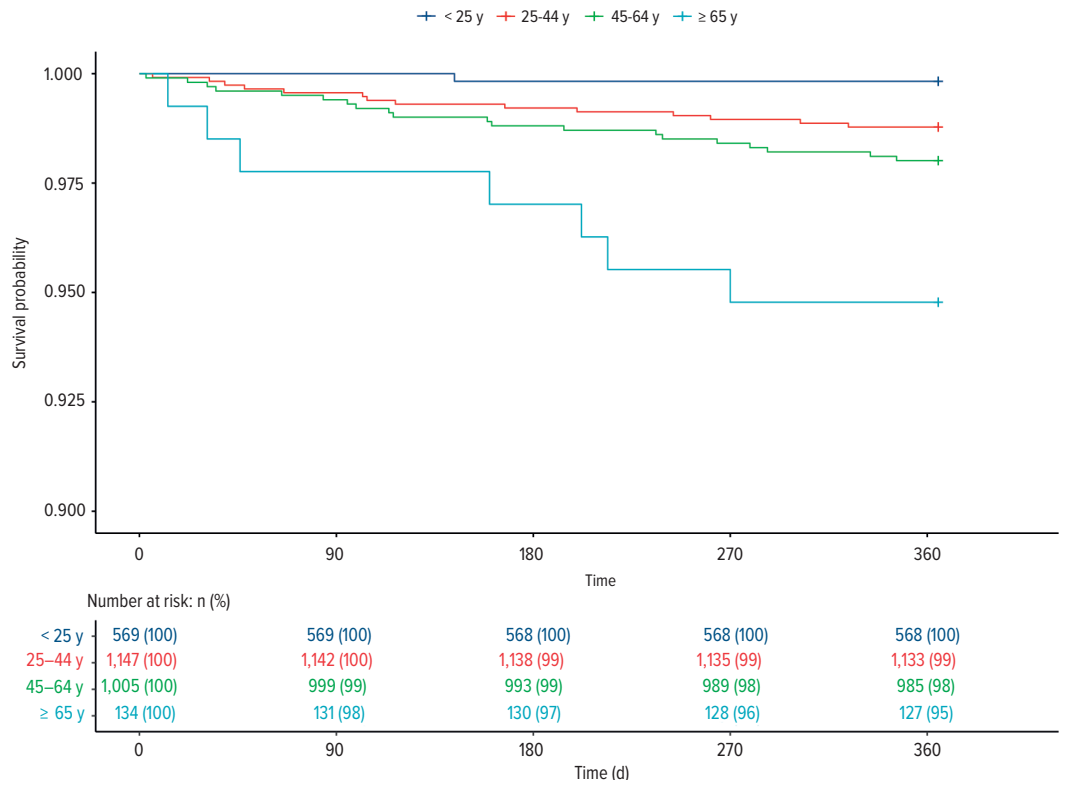


Table 2.

Association Between Clinical Factors and All-Cause or Suicide Mortality Within 1 Year

	Suicide mortality (n = 77)				All-cause mortality (n = 125)			
Factor	**HR (95% CI)[a]**	***P*[a]**	**Adjusted HR (95% CI)[b]**	***P*[b]**	**HR (95% CI)[a]**	***P*[a]**	**Adjusted HR (95% CI)[b]**	***P*[b]**
Age (ref = 25–44 y)								
< 25 y	0.19 (0.05–0.64)	<.01	0.22 (0.07–0.76)	.02	0.14 (0.04–0.48)	<.01	0.16 (0.05–0.52)	<.01
45–64 y	2.05 (1.19–3.51)	<.01	2.08 (1.21–3.56)	<.01	2.46 (1.55–3.90)	<.01	2.54 (1.61–4.02)	<.01
≥ 65 y	4.76 (2.43–9.29)	<.01	5.36 (2.72–10.54)	<.01	8.56 (5.15–14.21)	<.01	9.40 (5.61–15.75)	<.01
Male sex	1.92 (1.23–3.09)	<.01	1.79 (1.13–2.82)	.01	1.85 (1.30–2.63)	<.01	1.82 (1.26–2.61)	<.01
First attempt	0.90 (0.57–1.40)	.63			0.89 (0.62–1.26)	.50		
Inclusion location (ref = other)								
General hospital wards	2.13 (0.79–5.75)	.13	2.09 (0.77–5.64)	.14	1.01 (0.40–2.57)	.98	1.06 (0.42–2.71)	.90
Intensive care unit	1.06 (0.62–1.80)	.84	1.17 (0.68–2.00)	.57	0.76 (0.52–1.12)	.16	0.88 (0.60–1.31)	.54
Method of suicide attempt (ref = poisoning)								
Cutting or piercing	1.17 (0.54–2.57)	.69			1.25 (0.68–2.28)	.47	1.39 (0.43–4.46)	.58
Hanging	1.05 (0.38–2.90)	.92			0.82 (0.33–2.01)	.66	0.66 (0.15–2.91)	.59
Jumping from a height	1.59 (0.39–6.51)	.52			1.97 (0.72–5.36)	.18	2.79 (0.62–12.55)	.18
Drowning	2.05 (0.28–14.79)	.48			2.54 (0.62–10.31)	.19	1.25 (0.19–8.20)	.81
Use of a firearm	NC				NC		NC	
Suicide attempt with AAU	1.58 (1.00–2.48)	.05	1.23 (0.77–1.96)	.38	1.34 (0.94–1.91)	.10	1.04 (0.73–1.50)	.81
Suicide attempt with medication overdose	0.80 (0.47–1.37)	.43			0.72 (0.48–1.09)	.12	0.95 (0.28–3.29)	.94

[a]Hazard ratio and *P* value estimated by bivariate Cox model.
[b]Adjusted hazard ratio and *P* value estimated by multivariate Cox model.
Abbreviations: AAU = acute alcohol use, HR = hazard ratio, ICU = intensive care unit, NC = not calculated.

Factors Associated With the Risk of Death by Any Cause Within 1 Year

The same variables, ie, being less than 25 years old, being between 45 and 64 years old, being older than 64 years, and male sex, were associated with mortality from any cause within 1 year ($P < .05$) in the multivariable Cox model.

Sensitivity Analysis

The association between the risk factors identified and death by suicide was not modified after removing the subjects with an unknown cause of death: male sex (HR = 1.79 [1.13–2.81], $P = .01$), being less than 25 years old (HR = 0.22 [0.07–0.76], $P = .02$), being between 45 and 64 years old (HR = 2.07 [1.21–3.56], $P < .01$), and being 65 years of age or older (HR = 5.35 [2.72–10.53], $P < .01$). Second, among patients with a history of previous SA, 1,610 patients of 3,454 accepted the phone call within 10 days after the attempt. Accepting the phone call within 10 days does not appear to be associated with suicide deaths in bivariate and multivariate analysis (HR = 0.63 [0.34–1.17], $P = .14$; and HR = 0.55 [0.30–1.02], $P = .06$).

DISCUSSION

In this article, we presented a description of all causes of death in a cohort of 7,406 people who attempted suicide. Our study tried to determine the causes, rate of death, and associated factors within 1 year after a nonfatal SA. During the year of follow-up, of the 7,406 subjects admitted to the hospital after an SA, 1.7% died. Half of the deaths occurred within the first 4 months after the SA. Hanging and self-poisoning were the methods most often used for suicide. In addition, we demonstrated that male sex and being older than 44 years of age were associated with a higher risk of death by suicide at 1 year. In contrast, being younger than 25 years old was associated with a lower risk of death by suicide. Nonetheless, our results highlighted that a history of previous SAs, the method used for the SA, and the severity of prior SAs were not associated with death by suicide at 1 year. Finally, our cohort has almost the same distribution of socio-demographic characteristics (a higher percentage of women and an average age of 38 years) and clinical features (half were first-time attempters, few were serious attempts) as the other studies on natural cohorts of suicide attempters admitted to emergency departments or hospital.[3]

We estimated a rate of all-cause mortality of 1.7% at 1 year after an SA. Past studies exploring the outcomes of people who attempt suicide found an all-cause excess mortality in comparison to the general population and more specifically for deaths due to suicide, accidents, and cardiovascular diseases.[4,5,23] Premature mortality due to physical health conditions could be explained by the interaction of lifestyle risk factors and poorer physical health care in people who attempt suicide.[5,24] In addition, the current scientific literature seems to estimate higher mortality rates for people who attempt suicide admitted to emergency departments or hospitals, ranging from 2.6% to 6.8%.[25–27] Nonetheless, the mortality rates were higher for men and increased with age.

We determined that the incidence rate of death by suicide was 1.0% at 1 year after an SA. For patients admitted to emergency departments or hospitals after an SA, recent cohort studies found rates of death by suicide at 1 year ranging from 1.4% to 4.4%.[11,25–28] Moreover, in 2021, a meta-analysis found a rate of death by suicide of 2.8% (2.2–3.5) 1 year after a nonfatal SA.[3] Surprisingly, our results are lower than expected given the higher incidence of suicide in the Hauts-de-France region.[16] The lower rate of death by suicide in our study could be explained by the effectiveness of the VigilanS brief contact intervention for people who attempt suicide but also by the variety of inclusion locations, average younger age, and the slightly higher proportion of women in comparison to previous studies.[29] However, direct standardization based on a European population estimated a higher incidence of suicide (1,415 per 100,000 PYs), which remains low but comparable to other studies. In our study, about one-third of those who died by suicide used self-poisoning and approximately another third used hanging, but 29.9% of the suicide methods used remained unknown. In comparison, previous studies highlighted that violent means were most often used for completed suicide (in particular, hanging), and poisoning was used in less than one-third of cases.[30–32]

Regarding the most at-risk period, half of the deaths from all-cause mortality and suicide seemed to occur during the first 4 months after an SA. According to scientific literature, the same results were determined with the highest all-cause mortality rates in the first month following an SA.[4,25,26,32] This is partly explained by the fact that most suicide cases seem to occur within the first 6 months after an SA, with the highest risk occurring immediately after the SA.[10,25,32] We can hypothesize that the ongoing suicide crisis and the lack of medical care after an SA, particularly for depression, increase the risk of suicide.[33] In addition, a preexisting physical illness or functional disabilities may increase the risk of SAs, and/or dysregulation of the stress axis after an attempt may increase the risk of accidents or natural deaths.[4,23,34]

According to our results, adolescents had a lower risk of death by suicide within 1 year after an attempt, but people older than 44 years and males had a higher risk. Our results are in accordance with scientific literature, as past studies have already found that being an adolescent was associated with a lower risk of death by suicide after an SA and that older age and male sex were associated with an increased risk.[8,26,28,35–37] Moreover, male sex and older age were also identified as risk factors for all causes of death among people who attempt suicide, as we noted.[4]

Notably, our results did not find an association between the method used for the last SA or the severity of an SA and future death by suicide within 1 year. In comparison, a Swedish study determined that those who used hanging, strangulation, or suffocation as the SA method were at higher risk of later suicide.[31] Commonly, serious SAs and the use of violent methods for SAs (hanging, suffocation, strangling, and jumping from a height) were identified as risk factors for further suicides.[28,31,32,38–40] Our results may be due to the variety of our inclusion locations and the increased vigilance provided by our brief contact intervention program for those who attempted suicide with the use of a violent method or who had a serious SA.

In addition, while our study did not find an association between a history of previous SAs and death by suicide within 1 year, previous studies found a strong association with suicide repetition and subsequent death by suicide.[4,9,32] Nonetheless, a Spanish study published in 1999 found that the number of previous attempts increased the risk of reattempts but decreased the risk of subsequent suicide.[41]

Strengths and Limitations

One of the strengths of our study is the exhaustive analysis of causes of death through phone contact with general practitioners and/or the verification of death certificates for all persons who died within 1 year after an SA. Second, only 3% of causes of death remained unknown at the end of the study. The strong association between the risk factors identified and death by suicide was not modified by these subjects in the sensitivity analysis. Moreover, our study was based on an unselected cohort of people who attempted suicide in the Hauts de France region regardless of sex, age, SA method, or psychiatric diagnosis, which limits the selection bias. Finally, simple clinical features were assessed at inclusion, and their impact on eventual death by suicide was assessed. The identification of risk factors may allow the implementation of a targeted prevention of the population at risk: men over 45 years of age in brief contact interventions. In addition, greater vigilance is required for these patients at higher risk of death by suicide when the clinician initiates care after an SA.

One of the limitations of our study is that psychiatric diagnosis is not assessed at inclusion in the VigilanS program. Therefore, the strong association between the coexistence of a psychiatric disorder and an SA and the overall risk for suicide could not be analyzed. Second, we observed other causes of death besides suicide during the follow-up. Indeed, a death from another cause than suicide could precludes the occurrence of suicide. This may constitute a bias and competing risk models could have been used.

CONCLUSION

According to our findings, 1 in 100 people who attempted suicide died by suicide within 1 year after an attempt in the VigilanS program. Greater clinician vigilance is required in the first months following an SA, especially for male patients older than 45 years. While suicide is the most common cause of death after an SA,

the treatment of physical health conditions should not be neglected. The highlighting of simple sociodemographic and clinical factors associated with mortality is a first step in the improvement of current suicide prevention programs and is necessary to provide targeted interventions.

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Supplementary Material

Article Title: Suicide and All-Cause Mortality Within 1 Year After a Suicide Attempt in the VigilanS Cohort

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LIST OF SUPPLEMENTARY MATERIAL FOR THE ARTICLE

1. Table 1 Cause of Death Within 1 Year After an SA (N=125)
2. Figure 1 Study Flow Chart

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Supplementary materials

Supplementary Table 1. Cause of death within one year after an SA (N=125).

Cause of death	n (%)
Circulatory-system diseases	23 (19.5)
Neoplasms	6 (5.1)
Accidents	6 (5.1)
Infectious diseases	4 (3.4)
Unknown cause	4 (3.4)
Digestive diseases	2 (1.7)
Cause other than suicide	2 (1.7)
Suspected homicide	1 (0.8)
Suicide	77 (61.6)
Hanging	24 (20.3)
Poisoning	23 (19.5)
Unknown method of suicide	23 (19.5)
Jumping from a height	3 (2.5)
Drowning	1 (0.8)
Cutting or piercing	1 (0.8)
Use of a firearm	1 (0.8)
Road accident	1 (0.8)

Supplementary Figure 1. Supplementary material. Study flow chart