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FULL PAPER

Prevalence of hepatitis C virus estimates of undiagnosed individuals in different Italian regions: a mathematical modelling approach by route of transmission and fibrosis progression with results up to January 2021

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SUMMARY

This study provides an update on hepatitis C virus (HCV) estimates across Italy up to January 2021. A mathematical probabilistic modelling approach, including a Markov chain for liver disease progression, was used to estimate current HCV viraemic burden. Prevalence was defined by geographic area using an estimated annual historical HCV incidence by age, treatment, and migration rate from the Italian National database (ISTAT). Viraemic infection was estimated for the main HCV transmission routes by stages F0-F3 (patients without liver cirrhosis, i.e., potentially asymptomatic liver disease) and F4 (patients with liver cirrhosis, i.e., potentially symptomatic liver disease). By January 2021, we estimated that there were 398,610 individuals in Italy with active HCV infection (prevalence of 0.66%; 95% CI: 0.66-0.67), of which 287,730 (0.48%; 95% CI: 0.46-0.59%) were stage F0-F3. Prevalence values for all individuals with active HCV infection were: North 0.54% (95% CI: 0.53-0.54%), Central 0.88% (95% CI: 0.87-0.89%), South 0.72% (95% CI: 0.71-0.73%), and the Isles 0.67% (95% CI: 0.66-0.68%). The population at risk for previous/current drug injection accounted for 48.6% of all individuals with active HCV infection. A modelling approach such as this to estimate and update the prevalence of active HCV infection could be a useful methodology for the evaluation of healthcare policies related to HCV elimination plans.

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INTRODUCTION

Hepatitis C Virus (HCV) infection is the global leading cause of liver-related morbidity and mortality, and recent estimates report as many as 58 million individuals had chronic HCV in 2020 (Polaris Observatory HCV Collaborators, 2017; *World Health Organization: Hepatitis C* 2021; https://www.who.int/news-room/fact-sheets/detail/hepatitis-c). A high proportion of individuals do not show symptoms of the disease for decades, although the rate of disease progression increases with age (Hajarizadeh *et al.*, 2013; Sweeting *et al.*, 2006).

Since direct-acting antiviral drugs (DAAs) have become available for the successful treatment of HCV infection (Afdhal *et al.*, 2014; Sulkowski *et al.*, 2014), attention has focussed on identifying infected individuals. Only through the identification of this population will it be possible to achieve 2030 World Health Organization elimination targets (*WHO: Global health sector strategy on viral hepatitis 2016-2021*, 2016-2021; https://apps.who.int/iris/handle/10665/246177).

Historically, Italy has been considered the country with the highest rate of HCV prevalence in Western Europe (Bellentani *et al.*, 1999; Guadagnino *et al.*, 1997; Kondili *et al.*, 2021a; Maio *et al.*, 2000). However, a prevalence of active infection of around 1% (95% uncertainty interval: 0.4-1.4%) has recently been estimated using the "Polaris" model, which

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is grounded in the natural history of HCV progression and forecasts the HCV burden on the Italian general population (Blach *et al*., 2022). The reported number of patients treated annually, as tracked by the Italian Medicines Agency (*Agenzia Italiana del Farmaco*; AIFA; https://www.aifa.gov.it/aggiornamento-epatite-c) Monitoring Registry for DAAs, was allocated to the age and liver disease stage of the eligible population with HCV by the relative size of the population in each treatment disease stage (Kondili *et al*., 2018). A free screening program has now been implemented in Italy, with a specific fund approved by law, although decentralized HCV models are still being used without any uniform strategies across different regions (Kondili *et al.*, 2021a, 2020a). The coronavirus disease 2019 (COVID-19) pandemic has increased existing challenges in diagnosis and linkage to care of individuals with HCV (Blach *et al.*, 2021; Kondili *et al.*, 2021c; Mennini *et al.*, 2021). We have recently published national estimates on the number of individuals with HCV up to January 2020 by using a probabilistic approach to estimate the infection rate and a Markov model for liver disease progression in different Italian regions (Kondili *et al*., 2022a). The present analysis extends these findings further to January 2021, with the goal to update the HCV infection burden by considering the potential role of screening and diagnosis during the pandemic period.

MATERIALS AND METHODS

Study design

A previously validated mathematical model was used for this study (Kondili *et al.*, 2021b). The model was divided into two distinct computations. It computes the number of infected individuals on a national basis from available literature and the Italian National database ("Statistiche ISTAT", http://dati.istat.it/Index.aspx) by age, transmission route, and fibrosis stage from 1952 to January 2021 (Kondili *et al.*, 2022a, 2021b).

Study population and literature search

Data on HCV prevalence by route of infection in high risk groups was obtained from a literature search, as previously described elsewhere (Kondili *et al.*, 2021b).

The model

An evolutionary HCV transmission model was developed and implemented using the open-source programming language Python 3.7. The first stage of the model has been previously reported (Kondili *et al.*, 2021b), beginning in 1952 to the end of 2001 calculating the number of infected individuals by age, transmission route, and fibrosis stage. The evolutionary steps considered the insertion of new borns, new infections (the model accounts for six different transmission routes), possible fibrosis evolution (computed using a Markov chain approach), HCV treatment, and liver-related mortality rate. All cause-mortality was also considered (see below). After this stage, the number of individuals with HCV was subdivided for each region considering the distribution of the population and the variability of risk factors among the different regions as explained in the section "Transmission routes and associated risk". Subsequently, the evolution of the HCV transmission and liver disease progression among the Italian population was performed up to January 2021, with a specific method that included the migration of people (inter-regional and international), referred to as *regional infection burden evaluation*.

An overview of these steps is described below.

1) New-borns: The number of new-borns was added (data were derived from the Italian National database [ISTAT]) ("Statistiche ISTAT", http://dati.istat.it/Index.aspx).
2) Internal and external migration (i.e., the movement of people from one Italian region to another or from a foreign country to Italy, respectively): For internal migration, the number of individuals was included independent of age, whereas for external migration a minimum breakdown by age was considered using data derived from ISTAT ("Statistiche ISTAT", http://dati.istat.it/Index.aspx).
3) Individuals with HCV: Individuals recently contracting HCV per region, age, and year were considered (Kondili *et al.*, 2021b). The number of new HCV cases was then calculated following the infection probabilities, depending on age, transmission route ("SEIEVA - Sistema Epidemiologico Integrato dell'Epatite Virale Acuta", https://old.iss.it/web/guest/seieva/chi-siamo), and current year.
4) Treated patients: The estimated number of patients treated with anti-viral therapy (i.e., interferon- and DAA-based treatments) was subtracted from the population with HCV based on expert data provided by EpaC onlus Patient Association up to 2014 (https://www.epac.it/) and AIFA Registry data for DAA monitoring since 2015 ("Aggiornamento dati Registri AIFA DAAs - Epatite C cronica", https://www.aifa.gov.it/aggiornamento-epatite-c). Estimates from the Italian Platform for the Studies of Viral Hepatitis Therapies (PITER) cohort, for unclassified fibrosis stage by AIFA Registry, were also used (Kondili *et al*., 2021b, 2017). The average number of individuals successfully treated and subtracted were 2,000 every year from 1993 to 2000 and 7,000 every year from 2001 to 2014. The number of treated patients was based on AIFA Monitoring Registry for DAA (https://www.aifa.gov.it/aggiornamento-epatite-c)

data as follows: from 2015 to 2017 (mainly prioritized treatment criteria). An average of 36,296 patients each year was subtracted at a 95% rate of sustained virologic response from the number of total infected patients. Following universal treatment since 2017, a total of 56,499 treated patients for 2018, 36,348 treated patients for 2019, and 15,664 treated patients for 2020 were subtracted each year at a 98% rate of sustained virologic response from the total of infected patients.

5) Fibrosis evolution: Individuals with HCV who were considered to undergo a possible fibrosis progression according to a Markov chain probability evolution process (Kondili *et al.*, 2021b).
6) Mortality: The number of annual deaths per region and age provided by ISTAT ("Statistiche ISTAT", http://dati.istat.it/Index.aspx) was subtracted. In our model, we accounted for HCV-related mortality by considering the average values of transition probabilities following the F4 fibrosis stage reported by Linthicum *et al.*, 2016 (Linthicum *et al.*, 2016) and Kondili *et al.*, 2017 (Dienstag *et al.*, 2011; Kondili *et al.*, 2017; Townsend *et al.*, 2011; Wright *et al.*, 2006). All transition probabilities were adjusted for competing probabilities of death from other causes according to official data [ISTAT] ("Statistiche ISTAT", http://dati.istat.it/Index.aspx).

Transmission routes and associated risk

The model independently tracked each transmission route, distributing the weight of the effect of each route over time, as previously described (Kondili *et al.*, 2021b). Data for the following high-risk routes of HCV transmission were considered: previous or current drug injection, tattoos or body piercing, sexual transmission, glass syringe, blood transfusion, and vertical transmission. Key criteria for input of data in the model regarding age and year of infection were defined for each risk group. Based on HCV prevalence and the time-series of the Italian population, we reconstructed the probability of infection for ages 0-100, for years 1952 to 2021, and for the six different infection routes.

The total burden of infection at the beginning of 2002 was obtained using the results of the national phase (Kondili *et al.*, 2021b), divided for each macroregion. The criteria used to distribute the burden of infection across different macroregions are described below for each high-risk route of infection.

a) History of previous or current drug use – The distribution of previous or current drug use was estimated according to the 2002 national report on drug addiction (*Relazione Annuale Al Parlamento Sullo Stato Delle Tossicodipendenze in Italia 2002*, 2002; http://www.edscuola.it/archivio/handicap/tossicodipendenze02.pdf). Distribution of this risk factor was also based on the number of individuals with HCV in proportion to the number of individuals under treatment in a drug addiction service (SERD; *Servizi per le Tossicodipendenze*) according to fractions. Peak age of infection was 27 years, and HCV infection through injection of drugs was assumed to begin in 1970.

b) History of tattoo or body piercing – Nationally, ages of infection from tattoo or body piercing were between 15 and 70 years, with a peak age of 35 years (Kondili *et al.*, 2021b). However, data were not available to evaluate the prevalence of this route of infection across different regions; therefore, the number of individuals with HCV was subdivided proportionally to avoid errors in estimates. A region yields the number of those with HCV for a given age group proportionally to the number of those of that age in the region.

c) Sexual transmission – Due to the lack of available data for the route of sexual transmission, we applied the same formula as that for the route of tattoo and body piercing (Kondili *et al.*, 2021b). Transmission of HCV in this population occurred from 15 to 65 years of age (peak age of 35 years).

d) Previous glass syringe use – A higher prevalence was assumed to have occurred at an early age (0-8 years) due to glass syringe vaccination since 1950. However, neither infection by glass syringe (in 1975, the single use of disposable plastic syringes became law in Italy, substituting glass syringes nationwide) nor blood transfusion routes were considered in any new HCV cases since 2002; therefore, these routes can be referred to as "extinct" for the regional phase.

e) Risk of infection by blood transfusion in the past – Since blood transfusion was more likely to occur at older ages due to surgical intervention, the peak risk was centred at around 60 years of age. The age distribution profile started when the model began and peaked in the nineties (when the virus was finally isolated), and decreased gradually in subsequent years.

f) Vertical transmission – The risk of vertical transmission was calculated from the number of mothers with HCV; these females transfer the virus to new borns at a given percentage (values retrieved from ISTAT). The risk was estimated to be around 5.8% (Hofstraat *et al.*, 2017; "Statistiche ISTAT". http://dati.istat.it/Index.aspx). Reduction of the risk has been modelled with a linear decrease up to 0.015% (Kondili *et al.*, 2021b).

Markov chain progression for fibrosis evolution

The evolution from one stage to the next was modelled according to a Markov chain approach, previously described elsewhere (Kondili *et al.*, 2021b). Briefly, the following annual transition probabilities were used in the reference case: F0→F1=7.6%, F1→F2=9.5%, F2→F3=10.8%, and F3→F4=3.4%

(Linthicum *et al.*, 2016). It was assumed that not more than one transition per year could occur per patient, and potential spontaneous liver fibrosis regression was not considered.

Sensitivity analysis

Sensitivity analysis was performed using a Monte Carlo approach to estimate the effect of each input on the number of estimated individuals with HCV and their annual distribution among F0-F4 stages as described previously in detail (Kondili *et al.*, 2022a, 2021b). In brief, we repeatedly ran the model for 1,000 simulations, each time randomly picking the value of some input parameters (still around the values), and we assessed the effect of this randomization on the results of the model. Our Monte Carlo scheme intercepts two different types of randomization: risk distribution and transition probabilities. This approach considered the uncertainty in annual distribution among the F0-F4 stages, in the fibrosis annual transition probabilities, and the probability of self-curing (recover spontaneously without treatment). In each simulation, the self-curing and transition probabilities were derived (independently) from their predefined random distributions. We also conducted a sensitivity analysis of our model by transmission route for the different macroareas for individuals with stage F0-F3 and F4 (Kondili *et al.*, 2022a). Sensitivity analysis provides information on the margins for which prevalence estimates are valid, so as to understand the degree of variability in the data. The output of the Monte Carlo consisted of mean values of the different prevalence values computed using our model, together with a 95% confidence interval (CI), from 2.5 to 97.5 percent for each of them.

RESULTS

HCV prevalence estimates by Italian macroarea

The number of individuals with HCV in Italy in January 2021 was estimated at 398,610 (prevalence of 0.66%; 95% CI: 0.66-0.67%). Higher prevalence estimates were seen in regions of Central Italy (0.88%; 95% CI: 0.87-0.89%), such as Umbria (1%) and Marche (1%), followed by the South (0.72%; 95% CI: 0.71-0.73%), such as Basilicata (0.83%) and Calabria (0.81%), and the Isles (0.67%; 95% CI: 0.66-0.68%), in regions such as Sicily (0.66%) and Sardinia (0.71%); the lowest values were seen in regions in the North (0.54%; 95% CI: 0.53-0.54%), such as Piedmont (0.67%) and Veneto (0.6%) (*Table 1* and *Figure 1*). The distributions of HCV prevalence in potentially

Table 1 - *Estimates of the absolute number and percentage of viraemic HCV individuals in Italy according to fibrosis stage and macroarea up to January 2021.*

Macroarea/ fibrosis stage	*Absolute number and 95% CI*	*Prevalence (%) and 95% CI (%)*	*Percentage*
North			
F0-F3	121,533 (118,036-125,030)	0.43 (0.42-0.45)	42.2
F4	29,450 (26,073-32,827)	0.11 (0.09-0.12)	26.6
Total	150,983 (149,557-152,410)	0.54 (0.53-0.54)	37.9
Central			
F0-F3	70,028 (67,839-72,217)	0.59 (0.57-0.60)	24.3
F4	35,323 (33,388-37,257)	0.30 (0.28-0.31)	31.9
Total	105,351 (104,188-106,513)	0.88 (0.87-0.89)	26.4
South			
F0-F3	65,506 (63,448-67,564)	0.48 (0.46-0.49)	22.8
F4	33,422 (31,325-35,519)	0.24 (0.23-0.26)	30.1
Total	98,928 (87,623-100,233)	0.72 (0.71-0.73)	24.8
Isles			
F0-F3	30,663 (29,461-31,864)	0.47 (0.46-0.49)	10.7
F4	12,686 (11,473-13,898)	0.20 (0.18-0.21)	11.4
Total	43,348 (42,513-44,183)	0.67 (0.66-0.68)	10.9
Italy			
F0-F3	287,730 (279,911-295,549)	0.48 (0.46-0.59)	100
F4	110,880 (103,130-118,630)	0.18 (0.17-0.20)	100
Total	398,610 (396,960-400,260)	0.66 (0.66-0.67)	100

CI = confidence interval; HCV = hepatitis C virus.
Fibrosis stages: F0-F3 = asymptomatic; undiagnosed/unlinked to care and F4 = symptomatic; potentially linked to care and cure. Prevalence estimates were calculated according to each region's population by age group annually from 1952 up to January 1, 2021, from the Italian National Database (ISTAT).

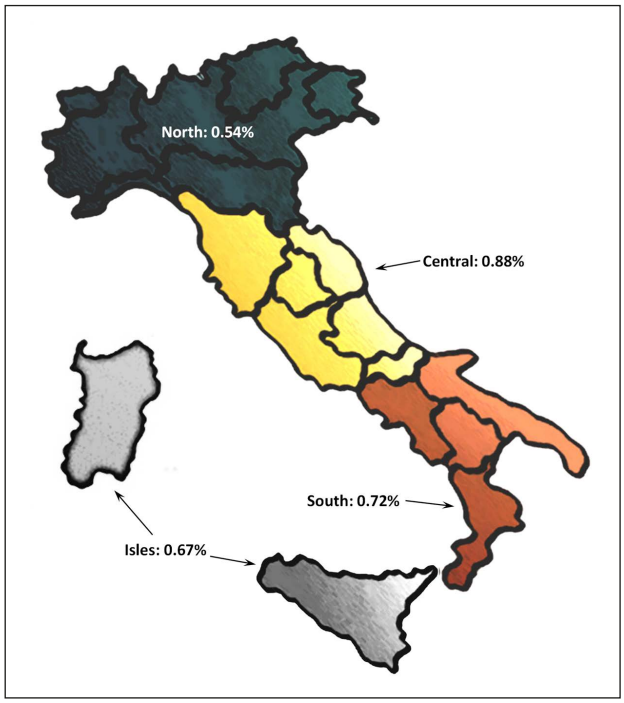

Figure 1 - *Map showing the estimated prevalence of active HCV infection in the 4 macroareas of Italy up to January 2021.*

asymptomatic individuals (stage F0-F3, i.e., patients without liver cirrhosis; estimated to be 287,730) and those with F4 stage disease (patients with liver cirrhosis, thus potentially symptomatic; estimated to be 110,880) for the 4 macroareas are summarised in *Table 1*. While the prevalence values for individuals with stage F0-F3 across the four macroareas were similar, varying from 0.43% to 0.59%, and similar to the overall prevalence for this stage (0.48%; 95% CI: 0.46-0.59%) (*Table 1*), prevalence values for individuals with stage F4 disease were generally two-fold lower than those with stage F0-F3, apart from the North, where it was approximately four-fold less (0.11% of F4 vs. 0.43% of those with F0-F3 fibrosis stage) (*Table 1*).

HCV prevalence by high-risk transmission route

Marked differences in overall prevalence estimates across the six high-risk infection routes were observed (*Table 2*). The highest prevalence in the general population was observed by previous or current drug use (0.32%; 95% CI: 0.32-0.33%) followed by previous or current tattoo use (0.17%; 95% CI: 0.16-0.17). Stratifying by macroarea showed little variation in prevalence estimates for each infection route (*Table 3*).

Table 2 - *Estimates of the absolute number and percentage of viraemic HCV individuals in Italy according to fibrosis stage and risk factor for route of transmission up to January 2021.*

Transmission route/fibrosis stage	*Absolute number and 95% CI*	*Percent of attributable risk and 95% CI (%)*	*Percentage*
Glass syringe			
F0-F3	9,516 (8,617-10,415)	0.016 (0.14-0.17)	3.3
F4	37,159 (35,511-38,808)	0.06 (0.06-0.06)	33.5
Total	46,675 (45,552- 47,799)	0.08 (0.08-0.08)	11.7
Transfusion			
F0-F3	4,037 (3,532-4,541)	0.007 (0.006-0.008)	1.4
F4	5,914 (5,347-6,482)	0.01 (0.009-0.011)	5.3
Total	9,951 (9,374-10,528)	0.02 (0.02-0.02)	2.5
*PWID**			
F0-F3	144,307 (139,845-148,768)	0.24 (0.23-0.25)	50.2
F4	49,404 (45,371-53,437)	0.082 (0.075-0.089)	44.6
Total	193,710 (192,186-195,235)	0.32 (0.32-0.33)	48.6
Sex			
F0-F3	42,141 (41,117-43,165)	0.07 (0.068-0.072)	14.6
F4	3,496 (2,915-4,078)	0.006 (0.005-0.007)	3.2
Total	45,637 (44,837-46,438)	0.08 (0.07-0.08)	11.4
Tattoo			
F0-F3	86,491 (84,161-88,822)	0.14 (0.14-0.15)	30.1
F4	13,681 (11,895-15,466)	0.023 (0.02-0.026)	12.3
Total	100,172 (98,922-101,423)	0.17 (0.16-0.17)	25.1

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Transmission route/fibrosis stage	Absolute number and 95% CI	Percent of attributable risk and 95% CI (%)	Percentage
Vertical			
F0-F3	1,237 (1,034-1,440)	0.002 (0.002-0.002)	0.4
F4	1,227 (997-1,458)	0.002 (0.002-0.002)	1.1
Total	2,464 (2,233-2,695)	0.004 (0.004-0.005)	0.6
All routes			
F0-F3	287,730 (279,911-295,549)	0.48 (0.46-0.59)	100
F4	110,880 (103,130-118,630)	0.18 (0.17-0.20)	100
Total	398,610 (396,960-400,260)	0.66 (0.66-0.67)	100

CI = confidence interval; HCV = hepatitis C virus; PWID = people who inject drugs.
Fibrosis stages: F0-F3 = asymptomatic; undiagnosed/unlinked to care and F4 = symptomatic; potentially linked to care and cure.
*Refers to previous or current risk of infection by drug use.

Table 3 - *Estimates of the absolute number and percentage of viraemic HCV individuals in Italy according to fibrosis stage, risk factor for route of transmission, and macroarea up to January 2021.*

Region	Transmission route	Fibrosis stage	Absolute number			Percent of attributable risk		
			Mean number	Lower 95% CI	Upper 95% CI	Mean (%)	Lower 95% CI	Upper 95% CI
North	Glass syringe	F0-F3	2,069	1724	2414	0.007	0.006	0.009
		F4	5,772	5051	6494	0.02	0.02	0.02
		Total	7,841	7176	8507	0.03	0.03	0.03
	Transfusion	F0-F3	936	742	1130	0.003	0.003	0.004
		F4	962	762	1163	0.003	0.003	0.004
		Total	1,898	1622	2174	0.007	0.006	0.008
	PWID	F0-F3	61,834	59693	63975	0.22	0.21	0.23
		F4	16,244	14395	18094	0.06	0.05	0.06
		Total	78,078	76951	79206	0.28	0.27	0.28
	Sex	F0-F3	18,567	17915	19218	0.07	0.06	0.07
		F4	1,278	943	1613	0.005	0.003	0.006
		Total	19,844	19255	20434	0.07	0.07	0.07
	Tattoo	F0-F3	37,600	36356	38843	0.13	0.13	0.14
		F4	4,798	3962	5634	0.02	0.01	0.02
		Total	42,397	41520	43275	0.15	0.15	0.15
	Vertical	F0-F3	529	368	689	0.002	0.001	0.003
		F4	396	256	535	0.001	0.001	0.002
		Total	924	733	1115	0.003	0.003	0.004
Central	Glass syringe	F0-F3	2,980	2593	3,368	0.03	0.02	0.03
		F4	12,494	11,932	13,055	0.11	0.10	0.11
		Total	15,474	14,962	15,986	0.13	0.13	0.13
	Transfusion	F0-F3	1,201	958	1,443	0.010	0.008	0.01
		F4	1,973	1,693	2,254	0.02	0.01	0.02
		Total	3,174	2,860	3,488	0.03	0.02	0.03
	PWID	F0-F3	35,458	34,145	36,770	0.30	0.29	0.31
		F4	15,502	14,393	16,611	0.13	0.12	0.14
		Total	50,959	50,208	51,711	0.43	0.42	0.43
	Sex	F0-F3	9,647	9,258	10,036	0.08	0.08	0.08
		F4	974	728	1,220	0.01	0.01	0.01
		Total	10,621	10,265	10,976	0.09	0.09	0.09

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Region	Transmission route	Fibrosis stage	Absolute number			Percent of attributable risk		
			Mean number	Lower 95% CI	Upper 95% CI	Mean (%)	Lower 95% CI	Upper 95% CI
	Tattoo	F0-F3	20,465	19,697	21,232	0.17	0.17	0.18
		F4	4,002	3,406	4,598	0.03	0.03	0.04
		Total	24,467	23,911	25,023	0.21	0.20	0.21
	Vertical	F0-F3	278	186	370	0.002	0.002	0.003
		F4	377	286	469	0.003	0.002	0.004
		Total	655	559	752	0.01	0.00	0.01
South	Glass syringe	F0-F3	3,220	2,828	3,611	0.02	0.02	0.03
		F4	14,048	13,304	14,792	0.10	0.10	0.11
		Total	17,268	16,601	17,934	0.13	0.12	0.13
	Transfusion	F0-F3	1,353	1,078	1,629	0.010	0.008	0.01
		F4	2,175	1,854	2,496	0.02	0.01	0.02
		Total	3,528	3,150	3,907	0.03	0.02	0.03
	PWID	F0-F3	32,384	31,077	3,3691	0.24	0.23	0.25
		F4	12,772	11,689	13,854	0.09	0.09	0.10
		Total	45,156	44,183	46,128	0.33	0.32	0.34
	Sex	F0-F3	9,266	8,824	9,707	0.07	0.06	0.07
		F4	837	633	1,040	0.006	0.005	0.008
		Total	10,102	9,682	10,522	0.07	0.07	0.08
	Tattoo	F0-F3	19,005	18,363	19,646	0.14	0.13	0.14
		F4	3,287	2,767	3,807	0.02	0.02	0.03
		Total	22,292	21,659	22,924	0.16	0.16	0.17
	Vertical	F0-F3	279	172	385	0.002	0.001	0.003
		F4	304	200	407	0.002	0.002	0.003
		Total	582	456	709	0.004	0.003	0.005
Isles	Glass syringe	F0-F3	1,247	979	1,515	0.02	0.02	0.02
		F4	4,845	4,415	5,275	0.08	0.07	0.08
		Total	6,093	5,653	6,532	0.09	0.09	0.10
	Transfusion	F0-F3	547	388	706	0.009	0.006	0.01
		F4	804	602	1,006	0.013	0.009	0.02
		Total	1,351	1,132	1,569	0.02	0.02	0.02
	PWID	F0-F3	14,631	13,896	15,367	0.23	0.22	0.24
		F4	4,886	4,236	5,536	0.08	0.07	0.09
		Total	19,517	18,850	20,184	0.30	0.29	0.31
	Sex	F0-F3	4,662	4,327	4,998	0.07	0.07	0.08
		F4	408	250	565	0.01	0.004	0.01
		Total	5,070	4,738	5,402	0.08	0.07	0.08
	Tattoo	F0-F3	9,422	8,822	10,023	0.15	0.14	0.16
		F4	1,593	1,289	1,898	0.02	0.02	0.03
		Total	11,016	10,494	11,538	0.17	0.16	0.18
	Vertical	F0-F3	153	82	223	0.002	0.001	0.004
		F4	150	74	225	0.002	0.001	0.004
		Total	302	214	391	0.005	0.003	0.006

CI = confidence interval; HCV = hepatitis C virus; PWID = (past or current) people who inject drugs. Fibrosis stages: F0-F3 = asymptomatic; undiagnosed/unlinked to care and F4 = symptomatic; potentially linked to care and cure.

Sensitivity analysis

Ranges in variability of estimates by fibrosis stage and age of cohort by sensitivity analysis were obtained as final results of the computations (up to January 2021). Variations around the mean values and CI ranges were computed by 1,000 Monte Carlo simulations. Specifically, the overall percentage variations around prevalence values were 0.42%-0.83%, for variations within 95% confidence intervals. The variations in 95% confidence intervals were 2.7-5.4% for F0-F3 and 7-14% for F4, indicating the robustness of our model's findings.

DISCUSSION

The present study confirms findings from our recent regional analysis of the prevalence of HCV across Italy up to January 2020 (Kondili *et al.*, 2022a, 2021b) and extends them to provide more recent estimates up to January 2021. Compared with our present findings, after 12 months (up to January 2021) and by using the same modelling approach, we have observed a slight decrease of 10,389 in the overall absolute number of individuals with HCV compared with estimates for the previous year (409,183 in January 2020 vs. 398,610 in January 2021). While the number of individuals with stage F0-F3 decreased from 300,171 to 287,981 (a decrease of 4.1%), the number of those with stage F4 (symptomatic) increased slightly, from 109,012 to 110, 813 (1,800 individuals and an increase of 1.6%).

The estimated number (and distribution across Italy) of patients with F4 cirrhosis is concerning and was not expected, considering their potential symptomatic disease and the high importance of viral eradication in these patients, who have been prioritized for treatment since 2015 when DAAs became available. The lack of early diagnosis in people with severe liver damage has also been documented by the AIFA DAA monitoring registry, which reported 20% of patients treated having a diagnosis of liver cirrhosis from 2019 to date ("Aggiornamento dati Registri AIFA DAAs - Epatite C cronica", https://www.aifa.gov.it/aggiornamento-epatite-c), patients that would have been diagnosed from 2015 in Italy. In addition, many people with viral hepatitis are still diagnosed too late (Lazarus *et al.*, 2019), often resulting in an increased risk of severe liver complications and death (Picchio *et al.*, 2021). Furthermore, several studies conducted in the opportunistic screening setting still suggest a high prevalence of individuals with active infection in severe stages of liver disease who have not yet been treated (Piazzolla *et al.*, 2021). These data imply that intervention focusing on case finding needs to be improved to ensure earlier diagnosis of populations at risk of disease progression. In terms of disease control, it is also important to increase our understanding of medical barriers that limit access to healthcare in different settings, starting from the general practitioner's awareness and clinical behaviour to patients with high risk of HCV infection and signs of chronic severe liver damage. Our findings indicate an estimated 110,880 individuals in need of urgent diagnosis, immediate linkage to care, and treatment to eradicate HCV infection to stop liver disease progression.

Considering the different factors for high risk of infection, people 46-55 years of age who previously injected or currently inject drugs and those who had undergone aesthetic procedures such as tattoos or body piercing were identified as the main populations in which higher prevalence of undiagnosed asymptomatic individuals (stage F0-F3 disease) is expected, with similar distribution across regions (Kondili *et al.*, 2021b). In these populations of potentially asymptomatic individuals, active HCV screening should be implemented across Italy to identify the submerged population of asymptomatic people with chronic HCV infection, as the required step toward achieving HCV elimination. This focused screening approach, if appropriately undertaken, would identify a high rate of individuals with HCV, due to the higher rate of infection related to risk factors in the younger population (drug use and aesthetic procedures at risk), and simultaneously reduce onward viral transmission by clearing HCV from those who are also at higher risk of spreading the infection (Kondili *et al.*, 2022b).

Although a higher estimated HCV prevalence of undiagnosed individuals was seen in some specific regions in Central Italy and in the Basilicata and Calabria regions in the South, similar but slightly lower prevalence estimates of undiagnosed individuals were generally observed across the four macroareas. This would suggest a decrease in the overall level of prevalence in HCV infection compared with the past in Italy, where higher HCV prevalence values and a gradient from North to South (3.9% in Veneto to 16.2% in Campania) was related mainly to the nosocomial transmission of infection (A.I.S.F, Associazione Italiana per lo Studio del Fegato, https://www.webaisf.org/per-il-paziente/le-malattie-del-fegato/; Guadagnino *et al.*, 1997). Despite using a different approach compared with the modelling used to estimate the global HCV burden, the results of this modelling study report an estimation within the range reported for overall active infection in Italy up to 2020. Specifically, an overall prevalence of 1% has been estimated up to 2020, with ranges varying from 0.4-1.4%, by the Polaris Observatory (Blach *et al.*, 2022), whereas this differential modelling of the HCV infection burden by transmission route granularity in Italy up to January 2021 shows a prevalence of active infection of 0.66% (0.66-0.67). These two independent estimates are slightly different suggesting that Italy is currently not a country with high

active HCV infection prevalence. However, in terms of achieving the elimination target, screening for active HCV infection is the only currently available tool for reaching asymptomatic individuals and ensuring their linkage to care and viral eradication. In this regard, despite these relatively low prevalence values compared with past values, the screening tools for the birth 1948-1988 cohort have been shown to be highly cost-effective in Italy (Kondili *et al.*, 2020a).
Analyses for the present study were performed up to January 2021, therefore including data up to 1 year after the severe acute respiratory syndrome coronavirus 2 virus pandemic (WHO, https://covid19.who.int/table). It is well documented that healthcare facilities across all Italian regions had to be re-organized in order to cope with the increased number of patients with COVID-19 admitted to emergency departments, resulting in the postponement of medical services and procedures considered "non-essential" or "deferrable". The reduction in the treatment rate seen in 2019 and the nearly complete interruption of DAA treatment during the COVID-19 pandemic are likely to impact directly on the management of HCV and are of serious concern (Blach *et al.*, 2021). A recent nationwide survey undertaken by the Italian Association for the Study of the Liver highlighted a significant decrease and suspension in the number of outpatient visits and prescriptions of antiviral treatment, which partially recovered 1 year later. However, the increase in outpatient visits was not followed by an increase in DAA prescriptions ("Aggiornamento dati Registri AIFA DAAs - Epatite C cronica", https://www.aifa.gov.it/aggiornamento-epatite-c; Aghemo *et al.*, 2020; Mennini *et al.*, 2021; Ponziani *et al.*, 2021). The potential impact of this deferral has been specifically assessed in a separate analysis, where it was estimated that deferring DAA treatment for only 6 months could increase the number of deaths from liver-related disease in HCV patients in Italy to more than 500 patients after 5 years (Kondili *et al.*, 2020b). Regions that have experienced a high number of cases of patients hospitalized for COVID-19, such as Lombardy, may be more susceptible to deferral in DAA treatment (Buoro *et al.*, 2020).

Limitations

The impact of other less-frequent transmission routes was not considered in the present analysis, and this limitation may have resulted in an underestimation of the observed prevalence values. Non-liver related mortality (i.e., natural mortality due to other causes) was based on ISTAT data (Italian Mortality Registry), which does not specify the cause of death and may have led to a potential overestimation of the population living with HCV infection (i.e., F4 stage disease). Another limitation is the high number of unregistered immigrants (potentially asymptomatic and undiagnosed for HCV) (Coppola *et al.*, 2019; Fedeli *et al.*, 2019; Massimo *et al.*, 2018) in Italy who were not considered in our estimations. Further studies are needed to explore the impact of the immigrant population on HCV prevalence.

CONCLUSION

Findings from the present analysis showed that the number of individuals with HCV in Italy was estimated at 398,610 in January 2021 (prevalence of 0.66%; 95% CI: 0.66-0.67%). Based on data derived from this modelling study, it is necessary to stress the importance of promoting attentive case finding and linkage to care in individuals with advanced liver disease (estimated to be 110,880) in addition to the active screening of asymptomatic people (estimated to be 287,730) in order to reduce prevalence, limit incidence, and potentially achieve the elimination of HCV in Italy.

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Conflicts of interest
LAK received teaching grants from AbbVie and Gilead. MA received funding for membership on Advisory Boards, for the preparation of educational materials, for research and educational grants, for membership on speaker panels and for support for travel to conferences from the following companies: Gilead Sciences, Janssen-Cilag, ViiV Healthcare, Merck Sharp and Dohme, Abbvie, Angelini, Pfizer, GSK, Menarini. AA serves on the advisory boards for AbbVie, Gilead, MSD, Intercept, Sobi, and Mylan and has received speaker fees from AbbVie, Gilead, Sobi, and Alfasigma. CMM received an advisor/speaker grant from AbbVie, Gilead, MSD, ViiV, Janssen-Cilag. AC has nothing to disclose. RM and VG are AbbVie employees and may own AbbVie stocks and options.

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