

Diagnostic gaps in HCV RNA testing in the era of elimination: a single-center study

Burcu Çalışkan Demirkiran¹, Neşe İnan², İrem Ataöv¹, Gülşen İskender¹, Duygu Mert¹, Semra Tunçbilek¹, Tuba Dal², Mustafa Ertek¹

¹ Department of Infectious Diseases and Clinical Microbiology, Dr Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, University of Health Sciences, Ankara, Türkiye

² Department of Medical Microbiology, Dr Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, University of Health Sciences, Ankara, Türkiye

Abstract

Introduction: It is essential to identify the current gaps in order to reach hepatitis C virus (HCV) elimination. This study focused on detecting undiagnosed HCV cases, to assess clinic awareness levels in the diagnostic process and explore strategies to decrease the number of undiagnosed cases.

Methodology: Anti-HCV seropositive patients identified at the hospital between 1 January 2022, and 31 December 2023, were retrospectively reviewed. Anti-HCV seropositive patients who did not have an HCV ribonucleic acid (RNA) test done were invited to the hospital, and those who came underwent HCV RNA testing.

Results: A total of 78,769 anti-HCV tests were conducted at the hospital, with 555 patients (0.7%) testing positive. HCV RNA testing was requested for 307 (55.3%) of the anti-HCV-positive patients during follow-up visits. The rate of HCV RNA test requests among anti-HCV-positive patients was significantly lower in surgical clinics compared to other clinics that requested HCV RNA testing ($p < 0.001$). Telephone calls were made to 248 patients who had not undergone HCV RNA testing, of whom 114 (46%) could not be reached. An anti-HCV signal-to-cutoff ratio (S/Co) ratio above 10.38 demonstrated 100% sensitivity and 89% specificity in predicting HCV RNA positivity (AUC: 0.95, $p < 0.001$).

Conclusions: Raising awareness among surgical clinics about HCV diagnosis and developing reflex testing algorithms to enable diagnosis at the patient's first hospital visit will significantly support efforts to eliminate HCV.

Key words: HCV screening; HCV RNA; reflex test.

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Introduction

Hepatitis C virus (HCV) infection remains a significant global concern regarding the development of cirrhosis and hepatocellular carcinoma [1]. An assessment of global HCV prevalence based on anti-HCV positivity estimated the number of cases to be 122 million in 1990 and approximately 184 million in 2005, with a prevalence rate of 2.8% [2]. A study conducted in 2020 reported the prevalence rate as 1.8% [3]. The countries with the highest prevalence were in Central Asia, East Asia, North Africa, and the Middle East; each exceeding 3.5% [2]. The seroprevalence of HCV infection in Turkey is low, ranging from 0.17% to 2.8%, with higher rates observed in the eastern part of the country [4,5].

The World Health Organization (WHO) has set targets for access to HCV diagnosis and treatment at 60% and 50% respectively by 2025, and 90% and 80% by 2030 [6]. The Coalition for Global Hepatitis Elimination (CGHE) began developing National Hepatitis Elimination Profiles in 2021 to assess the

status of national data, policies, and program progress. Data on hepatitis C prevalence, policy and program development, and progress toward WHO elimination goals are compiled in this health policy from the profiles of 33 countries and territories [7]. In Türkiye, the Viral Hepatitis Prevention and Control Program was released in 2018 [8]. However, due to the subsequent coronavirus disease 2019 (COVID-19) pandemic, the program could not be fully carried out. In order to meet the WHO HCV elimination targets, it is crucial to ensure that undiagnosed HCV cases have access to testing and that those infected with HCV can receive direct-acting antivirals.

It is recommended to perform screening with anti-HCV serology testing, and request HCV RNA testing if the initial test is positive. However, there are noticeable differences in the level of knowledge among clinics regarding HCV diagnosis and management [9]. This situation results in undiagnosed cases of HCV. Recognizing gaps in knowledge and increasing awareness among relevant clinics are essential to

decrease the number of undiagnosed patients.

The objective of this study was to identify patients with positive anti-HCV serology serological test results in the hospital and assess the clinics' awareness regarding further diagnostic tests and HCV diagnosis. Additionally, the study invited HCV seropositive patients who could not be diagnosed with HCV to the hospital and provided access to HCV RNA testing. By detecting HCV seropositive cases and undiagnosed HCV-infected patients, the study aimed to evaluate the feasibility of achieving WHO HCV elimination targets.

Methodology

Study design

The study population included patients who tested positive for anti-HCV from 1 January 2022 to 31 December 2023. Demographic and clinical details such as age, gender, department where the test was ordered, and results of the HCV RNA test (if available) were collected from the electronic health record system. Contact information for patients who did not have HCV RNA testing done was obtained from the hospital information system. These patients were contacted by phone and invited to the hospital for HCV RNA testing. The results of the HCV RNA tests for those who were contacted and visited the hospital were recorded.

Serological and molecular analysis

In this retrospective study, results of anti-HCV and HCV viral load tests obtained from the hospital information systems, between 1 January 2022 and 31 December 2023, were analyzed. Anti-HCV testing was performed using fully automated chemiluminescent immunoassay analyzers (Architect i2000 SR, Abbott, Illinois, USA; and Maglumi X3, Snibe Co. Ltd., Shenzhen, China) based on the chemiluminescence immunoassay (CLIA) method. HCV viral load quantification was performed using quantitative polymerase chain reaction (PCR), a nucleic acid amplification technique. HCV RNA levels in plasma samples from patients with confirmed anti-HCV positivity were measured by reverse transcription polymerase chain reaction (RT-PCR) using the Artus HCV RG RT-PCR kit (Qiagen, Hilden, Germany) on the Rotor-Gene platform.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS; IBM, Chicago, IL) version 23 software. Continuous data distribution was described by the median and interquartile range (IQR), while categorical data were summarized by

number and percentage. The Kolmogorov-Smirnov test assessed the normality of continuous variables. The Mann-Whitney U test compared continuous variables between two groups. The Chi-square test evaluated differences in categorical variables between groups. Diagnostic accuracy was assessed with receiver operating characteristic (ROC) curve analysis, and cutoff values were determined using Youden's Index. Statistical significance was set at $p < 0.05$.

Ethics approval

This study received approval from the Clinic Research Ethics Committee at the University of Health Sciences Dr. Abdurrahman Yurtaslan Oncology Health Administration and Research Centre, ensuring compliance with ethical standards and guidelines. (Dated 04/17/2025, with decision number 2025-04/60).

Results

A total of 78,769 anti-HCV tests were conducted at Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital between 1 January 2022 and 31 December 2023, and 555 patients (0.7%) tested positive. The median age of the patients was 59 years (range: 44–69), and 232 (41.8%) were male (Table 1). Of these, 317 (57.1%) were evaluated in 2022, and 238 (42.9%) in 2023.

When analyzed based on the type of clinic, 228 (41.1%), 184 (33.2%), 73 (13.2%), and 70 (12.6%) patients tested positive for anti-HCV in internal medicine, surgical, infectious disease, and gastroenterology clinics, respectively. HCV RNA testing was requested for 307 (55.3%) anti-HCV-positive patients during subsequent hospital visits. In 220 cases (71.7%), the requesting clinic also ordered

Table 1. Number of anti-HCV test requests and demographic information of patients who tested positive.

Variables	Value
Number of anti-HCV test requests	78769
Anti-HCV positive patient	555 (0.7%)
Age (years), median (IQR)	59 (44–69)
Gender, male (%)	232 (41.8%)
Anti-HCV titer, S/Co, median (IQR) (n = 504)	2.45 (1.47–6.04)
HCV RNA test request	
Yes	307 (55.3%)
No	248 (44.7%)
HCV RNA test result (n = 307)	
Positive	37 (12.1%)
Negative	270 (87.9%)
Clinics	
Internal	228 (41.1%)
Surgery	184 (33.2%)
Infection Disease	73 (13.2%)
Gastroenterology	70 (12.6%)

HCV: hepatitis C virus; IQR: inter quartile range; S/Co: signal-to-cutoff ratio.

HCV RNA testing, while in 87 cases (28.3%), another clinic made the request. HCV RNA testing was ordered for 175 (57%), 27 (8.8%), and 105 (34.2%) patients from the internal medicine, surgical, and infectious disease clinics, respectively. The rate of HCV RNA testing requests among anti-HCV-positive patients was lower in surgical clinics compared to the other clinics that requested HCV RNA testing. This difference was statistically significant ($p < 0.001$) (Table 2). Among the 307 patients who underwent HCV RNA testing, 37 (12.1%) had detectable HCV RNA levels. These patients received antiviral treatment.

The median anti-HCV signal-to-cutoff ratio (S/Co) ratio was 2.45 (1.47–6.04) in 504 patients tested with the Architect Anti-HCV assay. The remaining 51 patients were tested using the Maglumi X3 platform. Since S/Co ratios are assay-dependent and may vary between analytical platforms, only the results obtained with the Architect assay were included in the S/Co analysis. When comparing the anti-HCV S/Co ratio between HCV RNA-positive and HCV RNA-negative patients, the median ratio was 2.19 (1.4–5.18) in HCV RNA-negative patients, whereas it was 14.52 (12.72–15.21) in HCV RNA-positive patients, showing a statistically significant difference ($p < 0.001$) (Table 3).

HCV-RNA was used as the gold standard to identify the optimal threshold value in ROC curve analysis, which was determined to be 10.38 for the S/Co. The sensitivity was 100%, specificity was 89%, positive predictive value (PPV) was 59%, and negative predictive value (NPV) was 100% (Figure 1; Table 4).

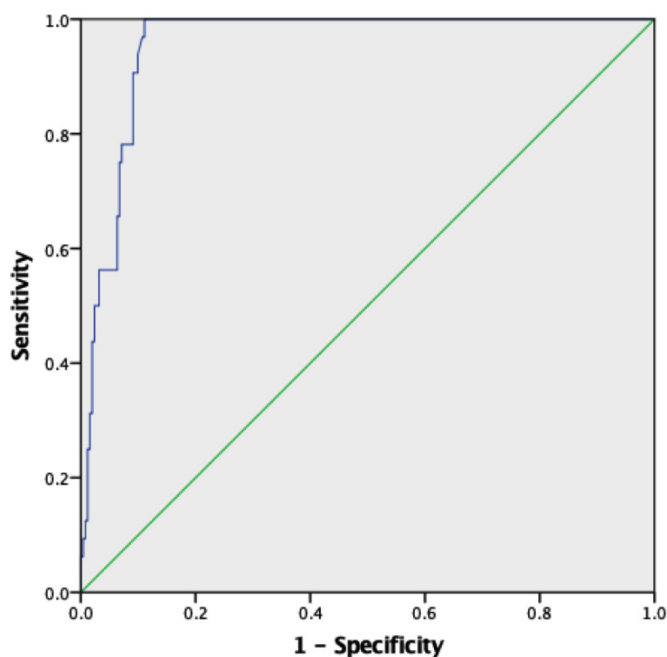
HCV RNA testing was not requested during subsequent hospital visits in 248 cases (44.7%). The patients' contact information were subsequently retrieved from the hospital's electronic information system, and their telephone numbers were obtained. It was not possible to establish contact in 114 (46%) cases. Five (2%) patients had died. A total of 129 (52%) patients were contacted by phone, informed of their anti-HCV positivity, and invited to visit the hospital.

Table 2. Comparison of clinics where HCV RNA was or was not requested from anti-HCV positive patients.

Variables	HCV RNA result		p^*
	No	Yes	
Year			
2022	152	165	$p = 0.08$
2023	96	142	
Clinics			
Internal	87	141	$p < 0.001$
Surgery	122	62	
Infection Disease	13	60	
Gastroenterology	26	44	
Total	248	307	

* Chi-square test was used for statistical analysis; HCV: hepatitis C virus..0

Figure 1. ROC analysis of anti-HCV S/Co values based on HCV RNA results.



Diagonal segments are produced by ties. ROC: receiver operating characteristic curve; HCV: hepatitis C virus; S/Co: signal-to-cutoff ratio.

Table 3. Comparison of anti-HCV titer levels based on HCV RNA test results.

Variables	HCV RNA negative	HCV RNA positive	p^*
Anti-HCV S/Co ratio	2.19 (1.4–5.18)	14.52 (12.72–15.21)	$p < 0.001$
Age, median (IQR)	60 (45–69)	51 (39–63)	$p = 0.049$

*The Mann-Whitney U test was used; HCV: hepatitis C virus; S/Co: signal-to-cutoff ratio; IQR: interquartile range.

Table 4. ROC analysis, positive predictive values, and negative predictive values of anti-HCV S/Co values based on HCV RNA results.

Anti-HCV S/Co	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUROC	95% CI		p
						Lower limit	Upper limit	
≥ 10.38	100	89	59	100	0.95	0.93	0.98	< 0.001

ROC: receiver operating characteristic curve; S/Co: signal-to-cutoff ratio; HCV: hepatitis C virus; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval; AUROC: area under the receiver operating characteristic.

Additionally, 15 (6%) patients reported having been tested at other medical facilities. Forty-four (17.7%) patients were invited to visit the hospital, where anti-HCV and HCV RNA tests were performed. Among these, 32 (12.9%) tested negative for anti-HCV, 12 were positive for anti-HCV, and 3 were HCV RNA positive. The combination of glecaprevir and pibrentasvir was planned for HCV-infected patients.

Discussion

This study assessed awareness among different clinics in further investigating patients with positive anti-HCV screening results. 55.3% of patients who tested positive for HCV underwent HCV RNA testing; most of the patients who did not receive HCV RNA testing were from surgical clinics. Among patients who tested positive for anti-HCV in the internal medicine, surgical, infectious diseases, and gastroenterology clinics, 61.8%, 33.7%, 82.2%, and 62.9% respectively had access to HCV RNA testing. WHO has set targets for access to HCV diagnosis and treatment at 60% and 50%, respectively, for 2025, and 90% and 80% for 2030 [6]. Although WHO elimination targets refer to the proportion of infected individuals diagnosed at the population level, the findings of this study provide insight into the diagnostic cascade within a single tertiary care center. In this study, approximately half of anti-HCV-positive patients underwent confirmatory HCV RNA testing, indicating that gaps in the diagnostic process still exist at the institutional level.

A 2017 study conducted at this center reported that HCV RNA testing was ordered for 52.63% of anti-HCV positive patients, with the lowest request rate coming from surgical departments [9]. During the 2022–2023 period, HCV RNA testing was requested in 55.3% of anti-HCV positive patients, showing a slight increase over the years; however, the overall pattern has remained largely unchanged. Similarly, it has been reported that HCV RNA testing could not be performed in approximately 33% to 50.16% of patients who tested positive for anti-HCV [10,11].

It is estimated that the global prevalence of chronic hepatitis C virus (HCV) infection was about 71.1 million, in 2015, and by 2020, this number had decreased to 56.8 million [12,13]. In a study in the southeastern part of the country, the seroprevalence rate among blood donors was 0.35% in 2011 and 0.16% in 2015 [14]. Along with global trends, Turkey has seen a decline in HCV seroprevalence over the years. In a study conducted by our center in 2017, the number of new HCV-infected cases was 55 per thousand, while in this study from 2022-2023, it was found to be 47 per

thousand [9]. Although the infection rate has decreased over the years, the elimination goal has not yet been reached [6].

The Infectious Diseases Society of America (IDSA) and the American Association for the Study of Liver Diseases (AASLD) recommend a one-time routine HCV screening for everyone over 18 years of age, and for those under 18 years who are at higher risk of HCV exposure [15]. In Türkiye, routine HCV screening is conducted during pregnancy, before surgery, and prior to marriage. The widespread screening programs used in the country should be effective. To ensure this, it is crucial to increase awareness and knowledge about HCV diagnosis and treatment. A national viral hepatitis prevention and control program is being carried out [8]. It includes various awareness-raising activities that aim to increase the knowledge of both people and physicians. However, these activities are only effective if they reach the intended audience. Conducting HCV awareness campaigns at surgical clinics will be an effective way to eliminate HCV.

Besides awareness campaigns aimed at patients and physicians, clinical and laboratory measures can also help eliminate HCV. They have the potential to simplify diagnostic processes, improve detection rates, and support linking patients to care. Point-of-care (POC) testing and dried blood spot (DBS) are two options that serve as alternatives to traditional HCV tests that use serum or plasma from venous blood for screening, diagnosis, and monitoring [16].

The studies suggest that reflex HCV Ag or HCV RNA screening for anti-HCV may help detect HCV-infected cases and decrease the number of undiagnosed cases [17,18]. Reflex viral load (VL) testing refers to a diagnostic strategy in which HCV RNA testing is automatically initiated following a positive anti-HCV antibody result. Two main approaches to reflex testing have been described: laboratory-based and clinic-based strategies. In laboratory-based reflex testing, when an anti-HCV test result is reactive, the laboratory directly performs HCV RNA analysis using the same blood sample or a duplicate specimen without requiring a new test request. In contrast, clinic-based reflex testing involves collecting an additional specimen for HCV RNA testing during the same clinical encounter after a positive anti-HCV rapid test result. Both approaches aim to complete confirmatory testing within a single patient visit [19]. The reflex viral load test has been shown to shorten the time to diagnose HCV, which can help reduce infection transmission [20]. WHO recommends the reflex viral load test as an additional strategy for elimination [19]. Most medical centers

require patients to make multiple visits, typically from two to five, to diagnose an HCV infection [21]. It is important to note that while some reflex tests can be performed with a single blood sample, most testing centers require two. Although the current situation may cause a decline in the use of reflex tests, encouraging the use of tests that require only one sample should be supported [20]. In this study, the HCV RNA test was not requested for about half of the HCV seropositive patients. When these patients were called to the hospital, roughly half of them had not been admitted. Reflex testing algorithms in a single hospital admission are effective in ensuring access to HCV diagnostic targets.

Among the patients who were recalled to the hospital, a considerable proportion tested negative for anti-HCV upon repeat testing. False-positive anti-HCV screening results may occur, particularly in samples with low S/Co ratios. In addition, differences between assay platforms and analytical variability may contribute to discordant results on repeat testing. This underscores the importance of confirmatory testing and standardized diagnostic algorithms in the interpretation of anti-HCV screening results. The Architect Anti-HCV test is a chemiluminescent microparticle immunoassay that qualitatively detects hepatitis C virus antibodies in serum and plasma samples. It has been reported that the Architect Anti-HCV test has a specificity of 99.6% and a sensitivity of 99.1%, with a 95% confidence interval. The anti-HCV test calculates a result based on S/Co and is used as a screening test for diagnosing HCV. Results with an S/Co value < 1 are interpreted as nonreactive and do not require repetition, while those with values ≥ 1 require at least two replicates and are reported as reactive [22]. The study's findings on anti-HCV reactivity for diagnosing HCV infection indicate that the most appropriate cut-off value is 12.27 [23]. One study demonstrated the effectiveness of the anti-HCV S/Co ratio in predicting non-viremic patients, suggesting a cutoff value of S/Co < 2.7 . It has been documented that no viremic patients had an S/CO ratio below 2.7, indicating that HCV-RNA testing is not recommended below this cut-off [24]. This study observed higher anti-HCV reactivity levels in patients infected with HCV. From a laboratory standpoint, and to ensure cost-effectiveness, facilities can develop an algorithm that uses a reflex testing protocol by establishing a specific cut-off value to lower the false positive rate in serological testing within the anti-HCV reflex HCV RNA testing algorithm.

WHO recommends strategies aimed at expanding access to HCV testing and improving linkage to care as

part of global elimination efforts. These include reflex HCV RNA testing following a positive antibody test, simplified diagnostic algorithms, and increased awareness among healthcare providers. Implementing such strategies can help reduce missed diagnoses and accelerate progress toward HCV elimination targets.

This study has several limitations. First, it was conducted in a single tertiary care center, which may limit the generalizability of the findings to other healthcare settings. However, the results provide valuable real-world insight into gaps in the HCV diagnostic cascade and highlight the importance of improving collaboration between clinical services and laboratories.

Conclusions

Identifying the barriers to HCV elimination is the first step toward achieving the goal of HCV elimination. This study observed differences in awareness of HCV diagnosis between clinics. It was noted that the awareness in surgical clinics should be increased. Clinical algorithms that ensure patient readmission to the hospital are not sufficient to reduce undiagnosed cases, since only about 50% of the patients were reachable. Therefore, diagnosing patients at their first visit and early detection of HCV-infected cases with reflex tests are crucial. Centers can develop cost-effective reflex test algorithms by setting their own cut-off values. In this study, an anti-HCV S/Co value of 10.38 was identified as having high sensitivity and specificity. Developing both clinical and laboratory algorithms is a vital step toward contributing to HCV elimination.

Corresponding author

Burcu Çalışkan Demirkiran, MD.

Department of Infectious Diseases and Clinical Microbiology, Dr Abdurrahman Yurtaslan, Ankara Training and Research Hospital, University of Health Sciences, Mehmet Akif Ersoy District Vatan Street Number 91, Ankara, Turkey Türkiye.

Tel: +90 546 534 90 91

Fax: 0 312 334 03 52

Email: drburcucaliskan@gmail.com

Conflict of interest

No conflict of interest is declared.

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