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Contents

Continuous Publication Volume 11 Number 3 November 5, 2021

SYSTEMATIC REVIEWS

META-ANALYSIS

LETTER TO THE EDITOR

World Journal of Clinical Infectious Diseases

Continuous Publication Volume 11 Number 3 November 5, 2021

Contents

ABOUT COVER

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SYSTEMATIC REVIEWS

Prevalence of anal human papillomavirus infection in patients with human immunodeficiency virus infection: A systematic review

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Abstract

BACKGROUND

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses nowadays.

AIM

To analyze the prevalence of HPV infection in human immunodeficiency virus (HIV)-positive patients and the risk factors associated with this infection through a review of studies published in the period from January 2010 to April 2020.

METHODS

A total of 384 articles were initially identified in our searches, of which ten were selected according to previously defined eligibility criteria.

RESULTS

Anal intercourse, absence of condom use, multiple partners, other specific sexual and life habits, and HIV infection are among the risk factors associated with anal HPV infection.

CONCLUSION

In general, there is a higher prevalence of anal HPV infection among HIV-positive patients, mostly in individuals over 30 years old, those with multiple partners, those who had an early homosexual debut, and cigarette, alcohol, and drug users.

Key Words: Human immunodeficiency virus; Anal human papillomavirus; Risk factors; Systematic review

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Core Tip: Human papillomavirus (HPV) is among the most common sexually transmitted viruses today. This systematic review aimed to analyze the prevalence of HPV infection in patients infected with human immunodeficiency virus (HIV) as well as the risk factors associated. Number of partners, absence of condom use, anal intercourse, multiple partners, sexual and life habits, and HIV infection are among the risk factors associated with anal HPV. There is a higher prevalence of anal HPV in patients infected with HIV, those with multiple partners, alcohol and drug users, and those with early age of first sexual intercourse with same-sex individuals.

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INTRODUCTION

Sexually transmitted infections (STIs) are among the most prevalent diseases, and human immunodeficiency virus (HIV) infection affects millions of people worldwide. In 2019, 1.7 million people were infected by HIV, which illustrates the relevance of this infection[1,2]. Another common STI is caused by human papillomavirus (HPV), which has a close relationship with the emergence of cancer in the perianal region, especially in individuals with weakened immune system, such as those who are seropositive for HIV[3].

The HPV is a DNA virus that causes a variety of benign and malignant lesions on the skin and mucous membranes[4]. More than 150 HPV types have been described, among which the HPV 16 and 18 are the most frequent and pathogenic types as they infect the genital tract and are associated with a high risk of oncogenesis.

In immunocompetent individuals, HPV is usually eliminated within 18 mo after infection, without clinical manifestations, and can be transmitted during this period. In addition, unprotected sexual intercourses, having multiple of partners, early initiation of sexual activity, and lack of immunization greatly contribute to the viral dissemination[4].

The most frequent clinical presentation of HPV is the cutaneous wart, with an estimated incidence of 7%-10% in the European population and of 1% in the American population. In immunosuppressed patients, these numbers are 50- to 100-fold higher, reaching more than 90% after 15 years of transplantation[5].

Another important feature is the association between HPV infection and carcinomas, among which cervical carcinoma is widely studied. However, this relationship is also well established in the anal region, with similarities between the lesions and the characteristics of the epithelia observed in those anatomical sites. Furthermore, the progression of the malignant lesions has similitudes between both regions as well[6-8].

HIV-seropositive patients have a higher risk of contamination by HPV because both infections have similar predisposing factors related to sexual habits. In addition, the persistence of HPV infection and a greater variety of HPV serotypes are observed in the HIV seropositive population[9,10].

The coinfection with HIV and HPV favors HPV DNA mutations, making this virus more pathogenic, regardless of the HIV viral load. The infection associated with immunosuppression can cause lesions with a higher carcinogenic potential, which implies greater aggressiveness of cell lesions and a worse prognosis[11,12].

The progression of HPV infection is established as the immunological damage associated with HIV infection progression. Papillomavirus persistence is inversely related to CD4+ T lymphocyte count and directly proportional to HIV load. In immunosuppressed people, the HPV recurrence rate becomes high[13,14].

In the general population, HPV-associated squamous cell carcinoma in the anal region is rare. However, anal cancer is more prevalent in HIV seropositive populations, men who have sex with men, and women with a history of cancer in the genital region[6,8,15,16].

Several studies have been pointing out the relationship between HIV and HPV, indicating a higher risk of papillomavirus infection in individuals with immune suppression. In this sense, it is essential to compile results of studies evaluating the coinfection with these viruses and demonstrating associated risk factors that contribute to the occurrence of anal changes by HPV. The aim of this systematic review was to verify the prevalence of anal HPV in HIV-seropositive patients as well as to list risk factors associated with this event.

MATERIALS AND METHODS

This is a systematic review conducted in accordance with the PRISMA recommendation (main items for reporting systematic reviews and meta-analyses).

Eligibility criteria

We selected articles on the prevalence and risk factors associated with HPV infection in anal and perianal sites in HIV-seropositive patients from January 2010 to April 2020 that were published in English, Portuguese, and Spanish.

Types of studies

Original research articles on clinical, experimental, and retrospective studies were included, and review articles, *in vitro* fertilization research, case series, and case reports were excluded. Articles that were not open access were not considered in this review.

Types of participants

The population chosen for this review was HIV- and HPV-seropositive patients, and all studies that only included individuals infected with HIV or HPV alone were excluded. We also excluded all articles that involved individuals under the age of 18.

Types of intervention

The articles that comprise the present review deal with the investigation of risk factors associated with HIV-seropositive patients with HPV coinfection at perianal site.

Information sources

The search for articles was conducted from March to April 2020, in the Scientific Electronic Library Online (SCIELO), United States National Library of Medicine (PubMed), and Virtual Health Library databases. In a strategy for searching data in all the bases, the following descriptors were used: HIV infections, papilloma virus infections, and anal canal.

Three authors independently carried out the selection of articles: Brandão CC, Ferreira IS, and Mota FS, co-authors of this paper. Then, duplicate articles were excluded and articles whose abstract did not address risk factors for HPV coinfection in anal/perianal sites in HIV seropositive patients were eliminated as well. Finally, the articles were fully read, including only those that addressed the topic of interest. The interpretative ambiguities and/or doubts were solved by consensus among the authors.

After reading the articles, the main data of each study were extracted, such as author(s), year of publication, factors associated with patients with viral coinfection, and prevalence data of the populations, described in Table 1.

Risk of bias analysis

Among the studies that compose this review, the cross-sectional ones have a greater risk of bias due to confounding factors, especially the lack of control methods used as statistical modeling. In cohort studies, the greatest risk of bias is identified as loss to follow-up. However, it is important to indicate that these biases were not able to significantly change conclusions, which can be verified through the coincident findings in the studies, regardless of the method used and the sample involved.

Table 1 Comparative summary of the articles studied

Ref.	Location	*n*	Study design	Predominant age	Predominant number of lifetime partners	Prevalence of anal HPV infection in HIV+ patients	Prevalence of anal HPV infection in HIV- patients
Li *et al*[17], 2016	China	889	Transversal	≤ 39 yr old: 79.53%	6-30: 58.71%	82.69%	62.81%
Wirtz *et al* [18], 2015	Moscow, Russia	124	Transversal	≤ 35 yr old: 82.1%	α ≥ 5: 64.5%	50%	30.30%
Hu *et al*[21], 2013	Beijing, China	671	Transversal	Median: 28 yr old	-	82.10%	57.50%
Lin *et al*[19], 2018	Taiwan	279	Transversal	< 30 yr old: 71%	< 10: 81.0%	85.30%	73.30%
Welling *et al* [25], 2015	Amsterdam	778	Prospective cohort	Median: 38 yr old	> 100: 64.42%	69%	45%
Wiley *et al* [24], 2013	United States	1262	Multicenter cohort	≤ 59 yr old: 71%	Average: 270	91%	70%
Zhang *et al* [22], 2014	Shenzhen, China	408	Transversal	20-39 yr old: 88.9%	Median: 3	71.40%	33.80%
Ren *et al* [26], 2017	China	164	Transversal	≤ 34 yr old: 91.4%	γ ≥ 1: 92.9%	81%	48.20%
Nowak *et al* [20], 2016	Nigeria	154	Transversal	≤ 29 yr old: 81.1%	≤ 50: 75.32%	91.10%	40.60%
Somia *et al* [23], 2018	Southeast Asia	392	Prospective cohort	≥ 30 yr old: 58.2%	Median: 42.5	89.80%	65.30%

α: Last 12 mo; γ: Last 3 mo. HPV: Human papillomavirus; HIV: Human immunodeficiency virus.

RESULTS

After applying the uniterms, 384 articles were found (4 in SciELO, 159 in PubMed, and 221 in BVS). Following the elimination of duplicate articles and analysis taking into consideration the eligibility criteria, ten articles were selected to compose the present review. Figure 1 shows the steps of this selection process.

All articles were published in English from 2013 to 2018, evenly distributed over this period.

Concerning the geographical areas where the studies analyzed were performed, there was a prevalence of studies conducted in Asia (70%), most of which were conducted in China. None of the studies that comprise this review was carried out in the Brazilian population.

Most articles (70%) used the cross-sectional methodological approach. All studies were conducted with groups of men who have sex with men (MSM) aged 18 years or older. The instruments used by the researchers included self-administered questionnaires and/or interviews that covered sociodemographic information and sexual behavior, which were used as variables associated with anal HPV infection. Most articles also assessed the relationship between the infection and lifestyle habits.

As for the sociodemographic aspects, no standardization of analyses such as age range and education level was verified within the selected studies. In 40% of the studies, most participants were less than 29 years old. Regarding education, half of the analyzed articles revealed a higher prevalence of individuals with complete or ongoing higher education.

The isolated or concomitant use of alcohol, cigarettes, and illicit drugs was little explored in the articles.

Regarding the number of homosexual partners of the participants in the studies which make up this analysis, less than half indicated data about the number of lifetime partners and a minority stratified this data into shorter periods (3 mo, 6 mo, and 12 mo prior to the date of the study).

As for the age of first sexual intercourse with same-sex partners, half of the studies did not analyze this characteristic in their populations. Moreover, the other half evidenced divergent results, partly indicating age over 18 and partly indicating age under 18.

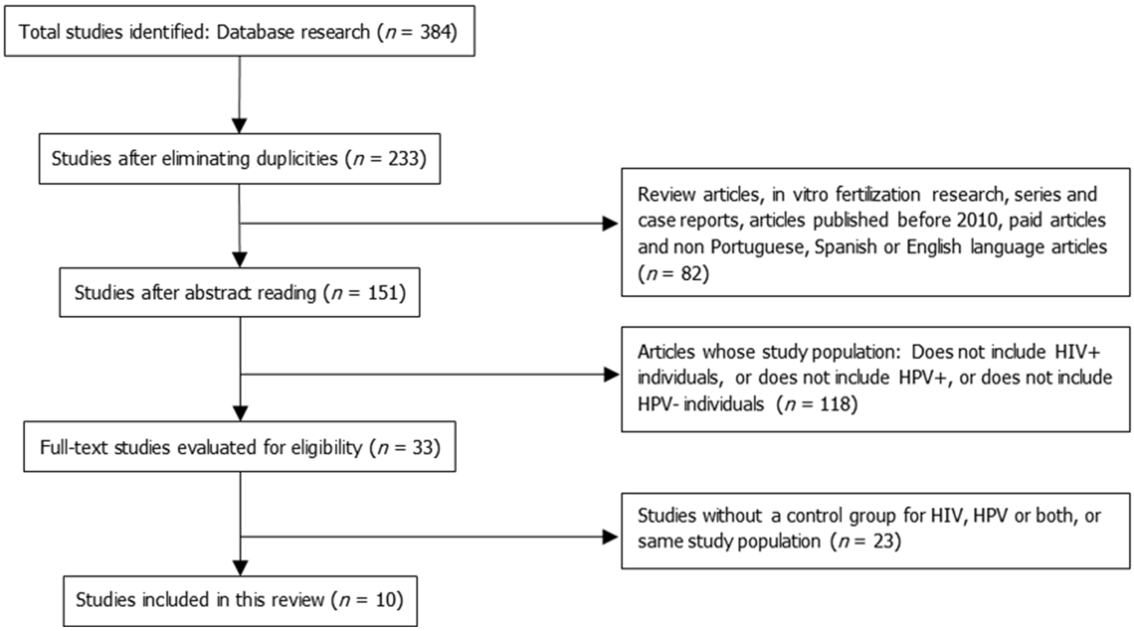

Figure 1 Flow chart of article selection. HPV: Human papillomavirus; HIV: Human immunodeficiency virus.

Overall, the studies were in agreement regarding the prevalence of anal HPV infection in HIV-seropositive patients, whereas the same levels of agreement were not found for anal HPV infection in HIV-seronegative patients (Table 1).

DISCUSSION

When analyzing the ten studies selected in this review, it was observed that none of them was conducted in Brazil. The methodological criteria listed for eligibility and the search mechanisms established here might have been responsible for this exclusion, especially concerning the non-inclusion of articles that were not open access.

Another important characteristic of this review was that most studies were cross-sectional. This methodological proposal is easier to execute, but it limits the evaluation of causality. The cause-and-effect relationship between the studied diseases is thus impaired, mainly because both diseases have similar epidemiological characteristics, and this kind of study does not offer a progressive perspective of the outcomes evaluated within the natural history of the disease.

In 40% of the studies[17-20], most patients were less than 29 years old. In the other studies, there was a wide variation in age classification, with two articles[21,22] reporting median ages of 28 and 29 years, respectively, and three articles[23-25] indicated a median age of greater than or equal to 30 years old.

Li *et al*[17], Wiley *et al*[24], and Ren *et al*[26] performed a more refined analytical approach in their studies, presenting more consistent results that indicate associations between age group and HPV infection. The research done by Wiley *et al*[24] demonstrated that patients aged between 40 and 69 years are more prone to infection by oncogenic high-risk HPV serotypes. The study by Ren *et al*[26], in its turn, showed that 78.6% of the MSM aged 35 years or older had anal HPV infection, a higher prevalence compared to all younger age groups. Li *et al*[17] showed that participants under the age of 29 had a 66.7% prevalence rate for HPV infection in the anal region.

The second point regarding socio-demographic criteria was education. The authors' reports diversified the approach to this assessment. The level of education of the population investigated by Li *et al*[17], Wirtz *et al*[18], Lin *et al*[19], Somia *et al*[23], and Ren *et al*[26] revealed a preponderance of individuals with completed or in training Higher Education. Conversely, Nowak *et al*[20] and Zhang *et al*[22] had the majority of individuals surveyed with lower educational levels.

The article by Lin *et al*[19] found a statistically significant association between high level of education and prevalence of anal HPV (P = 0.001). The studies by Li *et al*[17] and Somia *et al*[23] evaluated the educational level as a possible explanatory variable for the presence of high-risk oncogenic HPV but they found no statistically significant association. The other studies only described the participants' schooling. The lack of

standardization of the studies regarding the stratification of variables and the number of patients included hindered further inferential discussions.

Variables such as marital status, ethnicity, and skin color were only little explored in the studies listed in this review, which unfortunately does not allow us to make further inferences about them as possible risk factors associated with the occurrence of anal changes associated with HPV infection in HIV-positive individuals.

In the articles that make up this systematic review, little was explored about the relationship between smoking and use of illicit drugs and the presence of anal HPV infection. Furthermore, all the studies that assessed smoking found a higher frequency of tobacco use among HIV-positive individuals. Welling *et al*[25] demonstrated, through multivariate analysis, a relationship between HIV and HPV serological statuses and smoking. They have found that 23.5% of the HIV-seronegative and HPV-positive patients smoke (P = 0.467), whereas in seropositive patients for both viruses the percentage is 41.8% (P = 0.075). It demonstrates that smoking is a habit related to HPV and HIV infections, a finding reinforced in the study by Wiley *et al*[24], which reported that smoking habit is directly related to a higher prevalence of positive tests for low- and high-risk HPV (P = 0.04).

Regarding illicit drug use, Lin *et al*[19] and Hu *et al*[21] identified that individuals who use illicit drugs had a higher odds of HIV infection, HPV infection, or co-infection with both viruses.

The number of partners was a factor evaluated with regard to sexual habits. Three studies investigated the number of partners that respondents had throughout their lives. The study by Lin *et al*[19] used the parameters of less than 10 partners and 10 or more partners throughout life, and no statistically significant association was found regarding HPV prevalence. Nowak *et al*[20], on the other hand, found a significant relationship between having a greater number of sexual partners and the prevalence of high-risk HPV. The study showed that having a lifetime number of more than 50 partners is associated with a greater risk of infection (P = 0.02). Additionally, Li *et al*[17] demonstrated that individuals who had an above-30 lifetime number of partners had a higher risk of HPV infection (P = 0.02) than those who had up to 30 partners throughout their lives. Welling *et al*[25] found a statistically significant relationship between HIV seronegative participants who have reported over-500 lifetime number of partners and low-risk HPV infection (P < 0.001), with no significant association identified among participants without HIV infection.

The study by Wiley *et al*[24] observed a higher prevalence of HPV infection among men who had more than 30 partners throughout their lives (P < 0.05). Hu *et al*[21], Zhang *et al*[22], and Somia *et al*[23] indicated in their studies that the number of sexual partners that respondents ever had is an important factor for HIV and HPV infection but it should be noted that these studies found no statistically significant associations regarding this variable. All the other articles that comprise this review did not assess the relationship of lifetime number of partners with HPV infection; they did so by considering shorter periods of 6, 12, and 24 mo prior to application.

Another variable that has been evaluated is the age of initiation of sexual life among homosexual individuals. Li *et al*[17], Wirtz *et al*[18], Nowak *et al*[20], Somia *et al*[23], and Ren *et al*[26] assessed in their studies the age of sexual intercourse debut, but this parameter was not significantly associated with a higher prevalence of HPV infection in any of these studies.

Another factor evaluated was the practice of receptive and insertive anal sex. In the study by Lin *et al*[19], 82.1% of the subjects practiced receptive anal sex. Moreover, they have also found a higher prevalence of receptive anal sex among HPV-positive individuals than among HPV-negative people (91.3% *vs* 75.6%, P = 0.01). The study by Hu *et al*[21] revealed that 83.7% of the HIV-seropositive patients practiced receptive and insertive anal sex; in contrast, these sexual practices were reported by 58.2% of the HIV-seronegative patients (P < 0.01), and no statistically significant relationship was found between this variable and the prevalence of HPV in this study. Another important data observed by Li *et al*[17] was that 65.51% of individuals who always use condoms during anal intercourse had HPV infection. Somia *et al*[23] reported that HIV-seropositive individuals are more likely to use condoms, corresponding to 69.2% of the studied sample (P = 0.02), and no statistical significance was found in the association between this parameter and HPV prevalence.

The most important risk factor identified for HPV infection after analyzing the articles was HIV seropositivity. In all articles, HIV-seropositive patients had a higher prevalence of HPV infection than HIV-negative individuals. The study by Hu *et al*[21] described that immunosuppression due to HIV infection increases not only the risk of HPV infection, but also the persistence of infection and reactivation of latent infection status. This study also found that people at higher risk of acquiring HIV infection are

also at higher risk to be infected with HPV, since both infections are associated with high-risk sexual habits. The study by Ren *et al*[26] further demonstrated that HIV-seropositive MSM were 4.1 times more likely to be infected with any HPV serotype in the anal region compared to HIV seronegative MSM.

Two studies stood out for bringing very specific data on the topic. Lin *et al*[19] compared HIV-seropositive and HIV-seronegative individuals and found that HIV-positive individuals had infections with more than one HPV serotype. Likewise, Wiley *et al*[24] found that men who were not infected with HIV had a 1.8-fold higher odd of having infections with a single HPV serotype than HIV carriers.

In 20% of the articles analyzed, it was possible to observe that HIV-seropositive individuals had a higher risk for infection by high-risk HPV serotypes, especially serotypes 16 and 18. Wiley *et al*[24] found in their sample that 31% of the HIV patients were infected with HPV serotype 16 or 18, while, for HIV-negative patients, the percentage was 20%. The trial by Lin *et al*[19] attested that recent studies detected that HIV-seropositive men with HPV serotype 16 infection showed a higher affinity of the virus to the cells of the anal epithelium, which might be an excellent marker for predicting anal precancerous lesions. The results obtained by Wirtz *et al*[18] revealed that positivity for HPV 16 and 18 was associated with individual behaviors such as the number of male sexual partners, the greater number of stable partners with this same infection, and the use of lubricants incompatible with latex condom, which potentially facilitates exposure to HPV infection during anal intercourse.

All the studies listed here compared the prevalence of HPV infection in the anal region of HIV-seropositive and HIV-seronegative patients. The studies by Li *et al*[17], Wirtz *et al*[18], Hu *et al*[21], Somia *et al*[23], Wiley *et al*[24], and Welling *et al*[25] obtained similar results regarding the rates of HPV infection among HIV-positive and HIV-negative individuals, showing that this prevalence ranged from 18.6% to 24.6%, being higher for HIV seropositive people. In the same way, the studies by Nowak *et al*[20], Zhang *et al*[22], and Ren *et al*[26] also found a considerable difference between HIV-positive and HIV-negative individuals, but at higher levels, ranging from 32.8% to 37.6%. Only the study by Lin *et al*[19] found a smaller difference in anal HPV prevalence in HIV seropositive compared to seronegative individuals (12%). The studies showed statistical significance, except for the articles by Wirtz *et al*[18] and Welling *et al*[25], which did not indicate the significance test value for HPV infection.

After the analysis of the articles included in this review, it was possible to establish a concept map that traces the relationship between the presence of HPV infection and the risk factors associated with this infection (Figure 2).

The concept map shown here establishes the relationship between HIV and HPV infections, assessing several other risk factors in individuals with coinfection that may increase the prevalence of anal changes. Overall, this review has shown that individuals aged 30 years or older and with higher levels of education have been found to be more susceptible to HIV. The use of cigarettes, illicit drugs, having more sexual partners throughout life, starting sexual intercourses with same-sex partners at an earlier age, and performing receptive anal sex were also related to a greater propensity to HIV infection (represented by blue lines in Figure 2). As can be observed, the number of lines that focus on the HIV risk factor makes us infer that HIV seropositive patients have a higher prevalence of HPV infection, which have been represented by the red line in the Figure 2.

CONCLUSION

This review sought to address the HPV prevalence among HIV seropositive patients. After applying all methodological criteria, only ten articles were selected, and all of them addressed risk factors linked to a higher prevalence of HPV infection in individuals infected with HIV. We initially expected a greater number of articles, which may indicate the need for further studies on this theme.

As expected, after a thorough reading of the selected articles, this study reinforced the understanding that the most important factor for the presence of anal HPV infection is HIV seropositivity. The studies used here always compared this relationship between HIV-positive and HIV-negative individuals, but other factors that further increase the risk of HPV infection were also assessed, such as age, smoking, alcohol intake and use of illicit drugs, multiple partners, early age for homosexual debut, and number of homosexual partners.

The importance of studying HPV lies in the fact that it is an agent legitimately associated with the occurrence of cancer, including anal cancer. It becomes even more

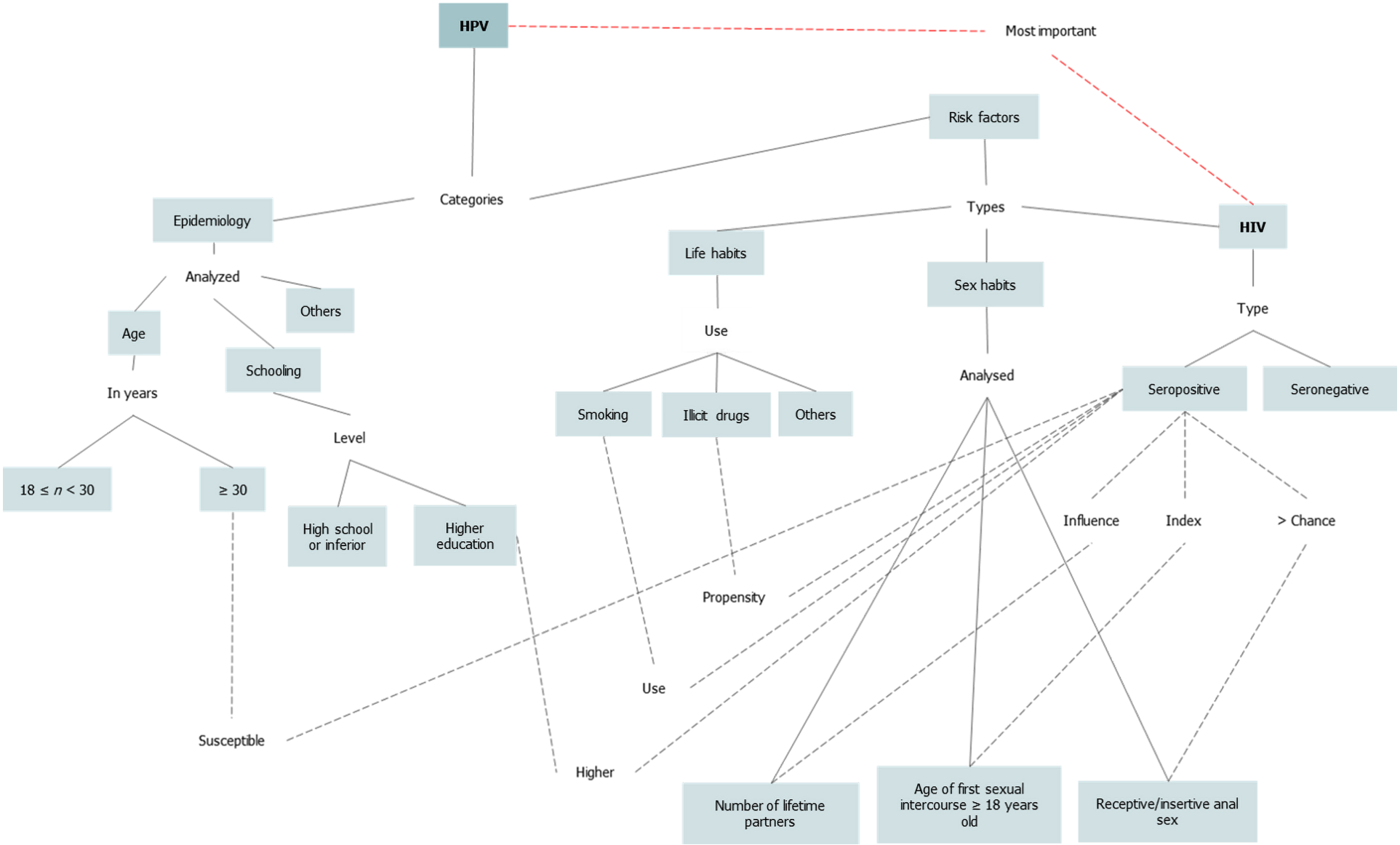

Figure 2 Conceptual map of the relations between human papillomavirus infection and the categories of analysis. HPV: Human papillomavirus; HIV: Human immunodeficiency virus.

important when there are individuals who are under a risk condition that greatly increases the chance of occurrence of this event, such as HIV-seropositive individuals. Thus, with regard to public health management, it is extremely important to recommend a close follow-up of these people, which can change the natural history of HPV disease. This review points out associated factors which in many cases can be modified, which has the potential to greatly contribute to reducing the incidence of high-grade lesions and anal cancer.

In addition, regarding overall patient management, it is possible to reduce the risk of infection by vaccination of groups that are more vulnerable to HPV-mediated high-grade lesions and cancer. The public health system in Brazil currently offers the quadrivalent vaccine for boys and girls aged 9 to 14 years, and this vaccination should be extended to groups of patients that are more susceptible to poorer HPV-related outcomes emphasized in this study.

The articles involved in this review lacked more refined statistical analysis and this decreased the possibility of making some inferences which were previously expected in the methodological planning process.

ARTICLE HIGHLIGHTS

Research background

Sexually transmitted infections are among the most prevalent diseases worldwide, which is considered a public health issue. In immunocompetent individuals, human papillomavirus (HPV) is usually eliminated within 18 mo after infection. However, several studies have been pointing out the relationship between the infection by HIV and HPV, indicating a higher risk of papillomavirus infection in individuals with immune suppression. HPV has also a close relationship with the emergence of cancer in the perianal region, especially in individuals with weakened immune system, such as those who are seropositive for HIV.

Research motivation

In the above-mentioned context, it is essential to compile results of studies evaluating the coinfection with these viruses and demonstrating associated risk factors that contribute to the occurrence of anal changes by HPV. Since HPV is an agent legitimately associated with the occurrence of cancer, in public health management, it is extremely important to recommend a close follow-up of these people, which can change the natural history of HPV disease.

Research objectives

This study aimed to explore the prevalence of anal HPV infection in HIV-seropositive patients as well as to list risk factors associated with this event.

Research methods

For this systematic review, PRISMA recommendation was followed. Articles on the prevalence and risk factors associated with HPV infection in anal and perianal sites in HIV-seropositive patients from January 2010 to April 2020 that were published in English, Portuguese, and Spanish were selected and analyzed.

Research results

Ten articles were selected, and all of them addressed risk factors linked to a higher prevalence of HPV infection in individuals infected with HIV. This study reinforced the understanding that the most important factor for the presence of anal HPV infection is HIV seropositivity.

Research conclusions

The analysis of the articles points to a higher prevalence of anal HPV infection in patients infected with HIV, those with multiple partners, alcohol and drug users, and those with early age of first sexual intercourse with same-sex individuals. Number of partners, absence of condom use, anal intercourse, multiple partners, sexual and life habits and HIV are among the risk factors associated with anal HPV infection.

Research perspectives

We initially expected a greater number of articles, which may indicate the need for further studies on this theme. This review points out associated factors which in many

cases can be modified, which has the potential to greatly contribute to reducing the incidence of high-grade lesions and anal cancer.

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META-ANALYSIS

Lymphocyte count predicts the severity of COVID-19: Evidence from a meta-analysis

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Abstract

BACKGROUND

In December 2019, coronavirus disease 2019 (COVID-19) was reported firstly in Wuhan, China. COVID-19 is currently a global pandemic.

AIM

To assess the suitability of lymphocyte count as a biomarker of COVID-19 severity.

METHODS

Five literature databases (PubMed/MEDLINE, Web of Science, Google Scholar, Embase, and Scopus) were searched to identify eligible articles. A meta-analysis was performed to calculate the standard mean difference (SMD) and 95% confidence interval (CI) of lymphocyte counts in coronaviral pneumonia cases.

RESULTS

Eight studies, including 1057 patients, were integrated in the meta-analysis. Lymphocyte counts were associated with severe coronavirus (CoV) infection (SMD = 1.35, 95%CI: 1.97 to 0.37, $P < 0.001$, $I^2 = 92.6\%$). In the subgroup analysis stratified by prognosis, lymphocytes were associated with CoV infection mortality ($n = 2$, SMD = 0.42, 95%CI: 0.66 to 0.19, $P < 0.001$, $I^2 = 0.0\%$), severity ($n = 2$, SMD = 0.93, 95%CI: 1.20 to 0.67, $P < 0.001$, $I^2 = 0.0\%$), and diagnostic rate ($n = 4$, SMD = 2.32, 95%CI: 3.60 to 1.04, $P < 0.001$, $I^2 = 91.2\%$).

CONCLUSION

Lymphocyte count may represent a simple, rapid, and commonly available laboratory index with which to diagnosis infection and predict the severity of CoV infections, including COVID-19.

Key Words: COVID-19; Lymphocyte count; Coronavirus; Severe of disease; Meta-analysis

Manuscript source: Unsolicited manuscript

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Core tip: Lymphocyte count reflects immune function and inflammatory state in infectious disease. Severe acute respiratory syndrome coronavirus 2 spreads and invades through respiratory mucosa, triggers a series of immune responses and induces a cytokine storm, resulting in changes in immune components such as lymphocytes. Previous studies have shown that the decrease in lymphocyte count can be used as an indicator of severity for both severe acute respiratory syndrome and Middle East respiratory syndrome; both of which are coronavirus (CoV) infections. Therefore, this systematic review and meta-analysis evaluate the diagnostic and prognostic utility of the lymphocyte count in patients with viral pneumonia by CoV infections.

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INTRODUCTION

In December 2019, in Wuhan, China, a novel CoV was identified as the causative agent of a novel pneumonia. The disease and the virus were subsequently termed coronavirus disease 2019 (COVID-19) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respectively, by the World Health Organization (WHO)[1,2]. To date, COVID-19 has infected over 465 000 people in 199 countries, with nearly 21 000 deaths [3]. These numbers continue to increase. As a CoV hypotype, SARS-CoV-2 is similar to the CoVs causing severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS)[4,5]. Both SARS and MERS spread rapidly worldwide, and have led to more than 10 000 human infections and 1000 deaths[4,6]. The largest epidemiological study of COVID-19 performed by the Chinese CDC showed that 13.8% of all COVID-19 cases were severe, and 4.7% were critical[7]. Critical patients, with a 49% case fatality rate, are at the most risk of death from COVID-19[7]. Therefore, it is important to develop a rapid, simple clinical method to identify severe COVID-19 cases.

Lymphocytes play a decisive role in the maintenance of immune homeostasis and the inflammatory response[8]. During the progression of an infectious disease, lymphocyte count reflects immune function and inflammatory state. SARS-CoV-2 invades *via* the respiratory mucosa, triggering a series of immune responses, including a cytokine storm, and affecting immune components, such as peripheral blood leukocytes and lymphocytes[9]. Consistent with this, studies have shown that lymphopenia, particularly the depletion of CD4 and CD8 lymphocytes, is a clinical characteristic of COVID-19 patients, especially those with severe infections[10-12]. Indeed, previous studies have shown that the decrease in lymphocyte count can be used to indicate the severity of SARS and MERS, both of which are CoV infections[13, 14]. However, due to the lack of analytical data, as might be provided by case–control or cohort studies, it is unclear whether lymphocyte count can also be used to reflect COVID-19 severity. Although Tan *et al*[15] had reported that lymphocyte count predicted the severity of COVID-19, this study had too few samples. However, relying on the association of CoV infection, we found some evidence in a small number of observational studies of COVID-19. Here, we performed a systematic review and meta-analysis to evaluate the diagnostic and prognostic utility of lymphocyte count in patients with viral pneumonia caused by CoV infections. Our aim was to explore the possibility that lymphocyte counts can predict COVID-19 severity and provide associated evidence.

MATERIALS AND METHODS

Search strategy and selection criteria

This protocol was registered with the PROSPERO international prospective register of systematic reviews (CRD42020177132, available from: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=177132) and followed the recommendations

established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[16]. We conducted a systematic review across five literature databases: PubMed/MEDLINE, Web of Science, Google Scholar, Embase, and Scopus. The search terms used were as follows: "lymphocyte count," and "pneumonia, viral" or "SARS" or "MERS" or "COVID-19". All of the searches were concluded by March 21, 2020, and two researchers independently evaluated the search results.

Eligibility criteria

We included published peer-reviewed articles describing case–control, cohort, or cross-sectional studies, where lymphocyte counts were measured in peripheral blood samples from humans of any age with CoV infections. Case reports, conference abstracts, and review articles were excluded.

COVID-19 infections were diagnosed using next-generation sequencing or real-time reverse transcription polymerase chain reactions (RT-PCRs)[17]. MERS-CoV was diagnosed according to the WHO criteria: a confirmed case was defined as a suspected case that was positive for MERS-CoV based on RT-PCR results[18]. SARS infections were confirmed based on a definite exposure history, as well as either a positive RT-PCR test during acute infection or detectable CoV-specific antibodies during convalescence[19]. We excluded studies conducted exclusively in patients with active cancer, chronic liver disease, HIV, or immunosuppression. When an article reported duplicate information from the same patient, the reports were combined to obtain complete data, but the case was only counted once.

Study selection

All of the titles and abstracts returned by the database search (Figure 1) were reviewed by first author (Zhao YS) independently to assess the need for a full-text review. Any disagreements were resolved through discussion between the same author and another author (Yu YX). Reasons for exclusion were recorded.

Assessment of risk of bias

Publication bias was assessed using a funnel plot, Begg's test and Egger's test.

Statistical analysis

According to a study by Hozo *et al*[20], we estimating the mean and standard deviation from the median and range, and the size of a sample as data extrapolation. When sample size was small (n < 25), we used a simple formula: x = (a + 2m + b)/4 to estimate the mean (x) using the values of the median (m), low and high end of the range (a and b, respectively). As soon as sample size exceeded 25, the median itself was the best estimator. When sample size was small (n < 15), we used the formula: S^2 = 1/12 × (((a - 2m + b)2)/4 + (b - a) 2) to estimate the standard deviation (S^2). When the sample size increased (15 < n < 70), Range/4 was the best estimator for S^2. For large samples (n > 70) Range/6 was the best estimator for S^2[20]. To indicate the severity of CoV infection, we designed a cohort that combined three different measures of prognosis with respect to the control group: diagnosed *vs* nondiagnosed, severe *vs* nonsevere, and death *vs* survival. The control group included nondiagnosed, nonsevere and survival groups. The case group was severe, including diagnosed, severe and death groups.

We used the random-effects model to calculate the standard mean difference (SMD) and 95% confidence interval (CI) for lymphocyte count in the CoV-infection patients and to draw a forest plot. Subgroup analysis was performed based on the study definition of severity. Heterogeneity between pairs of studies was quantified using the I^2 statistic. We investigated potential sources of heterogeneity, including prognosis and data source (original data *vs* extrapolated data), by performing subgroup analyses. One was prognosis subgroup that was divided into mortality, severity and diagnostic rate subgroups according to different prognosis investigated in included studies. One was data source subgroup that was divided into original data and extrapolated data subgroups. The criterion was whether the data extrapolated according to the study by Hozo *et al*[20]. All of the analyses were performed using Stata version 14 (Stata Corp., College Station, TX, USA).

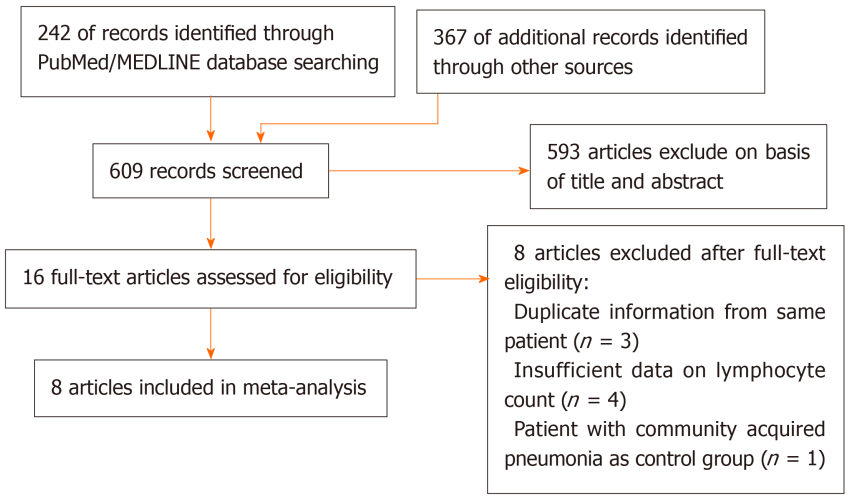

Figure 1 Article selection process.

RESULTS

Results of the electronic search

We identified 609 articles using database searches. After screening, 16 articles remained. After examination of the full-text articles, we excluded eight of these articles: three contained duplicate information from same patient; four articles had insufficient data on lymphocyte count; and one article chose a patient with community-acquired pneumonia as a control group. Therefore, eight studies were included in our meta-analysis[10,13,14,19,21-24].

Characteristics of the included studies

The characteristics of the eight included studies are summarized in Table 1. All of the studies included were prospective or retrospective case–control studies, cohort studies, or cross-sectional studies. The patient population in each study ranged from 30 to 346 (1057 patients in total). The earliest publications were from 2003[22,24], and the most recent article was published in 2020[10,21,23]. Most studies were based in China (n = 7), including Taiwan (n = 3)[13,19,24], Wuhan (n = 2)[10], Beijing (n = 1)[22], and Shanghai (n = 1)[23]. One study was based in Saudi Arabia[14]. The CoV diseases studied were COVID-19 (n = 3; 37.5%)[10,21,23], MERS (n = 1; 12.5%)[14], and SARS (n = 4; 50%)[13,19,22,24]. Four studies (50%) investigated prognosis with respect to diagnostic rate (diagnosed *vs* nondiagnosed)[19,22-24], two studies (25%) investigated prognosis with respect to severity (severe *vs* nonsevere)[10,21], and two studies (25%) investigated prognosis with respect to mortality (death *vs* survival)[13,14].

Characteristics of lymphocyte count

The means and standard deviations of lymphocyte counts from four studies[10,14,21,23] were extrapolated from sample size, median, and IQR (Table 1). In the forest plot, if the SMD (95%CI) was < 0, it showed that the mean and standard deviation of lymphocyte counts in the case group were less than those in the control group. That meant lymphocyte counts were less in severe CoV infection. Analysis showed that lymphocyte counts were associated with severe CoV infection (SMD = 1.35, 95%CI: 1.97 to 0.37, P < 0.001, I^2 = 92.6%) (Figure 2). There was heterogeneity among studies. Therefore, subgroup analysis, stratified by prognosis and data source, was performed. In the subgroup analysis stratified by prognosis, lymphocytes were associated with mortality due to CoV infection (n = 2, SMD = 0.42, 95%CI: 0.66 to 0.19, P < 0.001, I^2 = 0.0%), with severity of CoV infection (n = 2, SMD = 0.93, 95%CI: 1.20 to 0.67, P < 0.001, I^2 = 0.0%), and with the diagnostic rate of CoV infection (n = 4, SMD = 2.32, 95%CI: 3.60 to 1.04, P < 0.001, I^2 = 91.2%) (Figure 3). In the subgroup analysis stratified by data source, lymphocytes were associated with both the extrapolated data (n = 4, SMD = 0.92, 95%CI: 1.33 to 0.51, P < 0.001, I^2 = 64.0%) and the original data (n = 4, SMD = 1.97, 95%CI: 3.35 to 0.60, P < 0.001, I^2 = 96.5%) (see Supplementary Figure 1, which illustrates the forest plot of subgroup analysis stratified by data source), explaining the observed overall heterogeneity among studies. In addition, because few studies were included in our analysis, it was unclear whether the funnel plot was symmetrical

Table 1 Characteristics of the included studies

Ref.	City, country	Coronavirus disease	Outcome	*n* (case)	Lymphocyte count of case, mean ± SD (× 10^9/L)	*n* (control)	Lymphocyte count of control, mean ± SD (× 10^9/L)
Das *et al* [14], 2015	Saudi Arabia	MERS	Mortality	19	18.25 ± 11.75	36	24.00 ± 19.75
Chang *et al* [13], 2006	Taiwan, China	SARS	Mortality	73	0.81 ± 0.38	273	1.02 ± 0.48
Wang *et al* [10], 2020	Wuhan, China	COVID-19	Severity	36	0.80 ± 0.10	102	0.90 ± 0.10
Zhang *et al* [21], 2020	Wuhan, China	COVID-19	Severity	58	0.70 ± 0.13	82	0.80 ± 0.10
Cui *et al*[22], 2003	Beijing, China	SARS	Diagnostic rate	38	0.91 ± 0.44	200	3.00 ± 1.00
Chen *et al* [19], 2006	Taiwan, China	SARS	Diagnostic rate	15	0.60 ± 0.30	15	2.00 ± 0.20
Li *et al*[23], 2020	Shanghai, China	COVID-19	Diagnostic rate	10	1.22 ± 0.20	30	1.75 ± 0.33
Chen *et al* [24], 2004	Taiwan, China	SARS	Diagnostic rate	8	0.90 ± 0.30	62	1.50 ± 1.10

SARS: Severe acute respiratory syndrome.

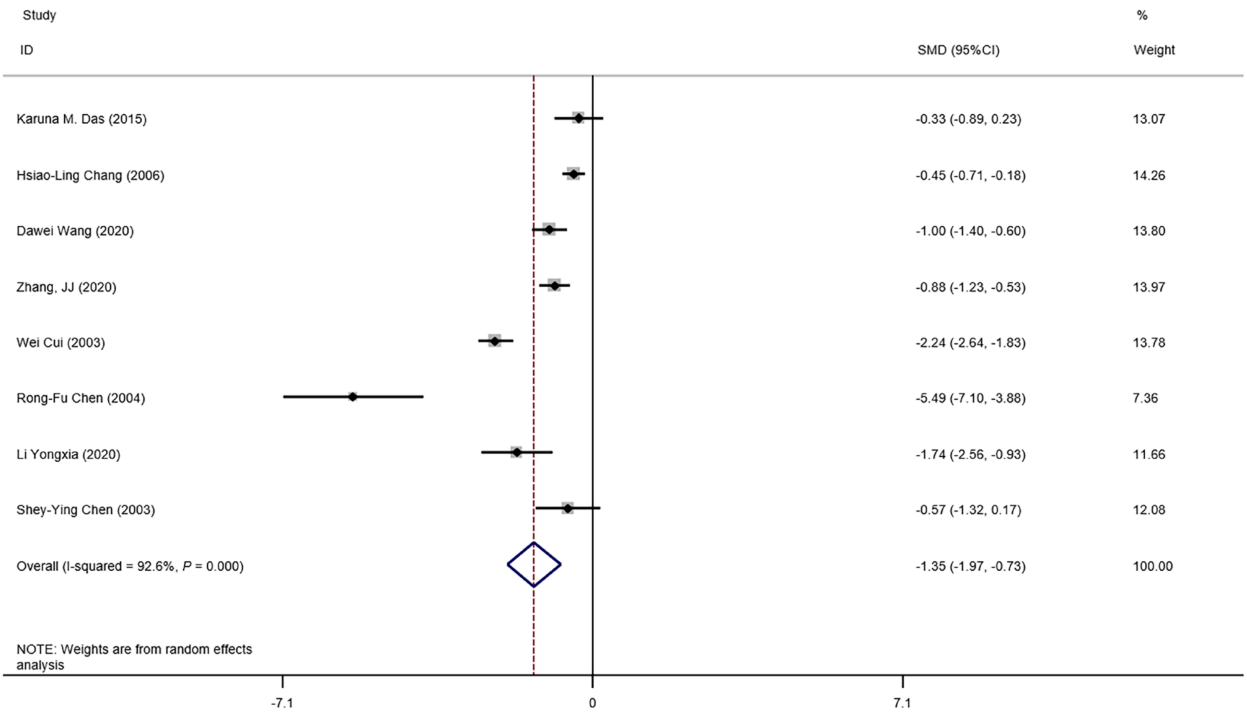

Figure 2 Forest plot: overall identification of lymphocyte count in patients with coronaviral pneumonia. P = 0.000 means P < 0.001. CI: Confidence interval.

(Figure 4), but Begg's test and Egger's test showed that publication bias had no significant effects on the results of the meta-analysis (P = 0.174).

DISCUSSION

COVID-19 is rapidly infectious and highly severe, with a high mortality rate[3]. Patients with COVID-19 exhibit a wide range of variability in disease severity. In clinical practice, we believe that low levels of lymphocytes are disadvantageous for

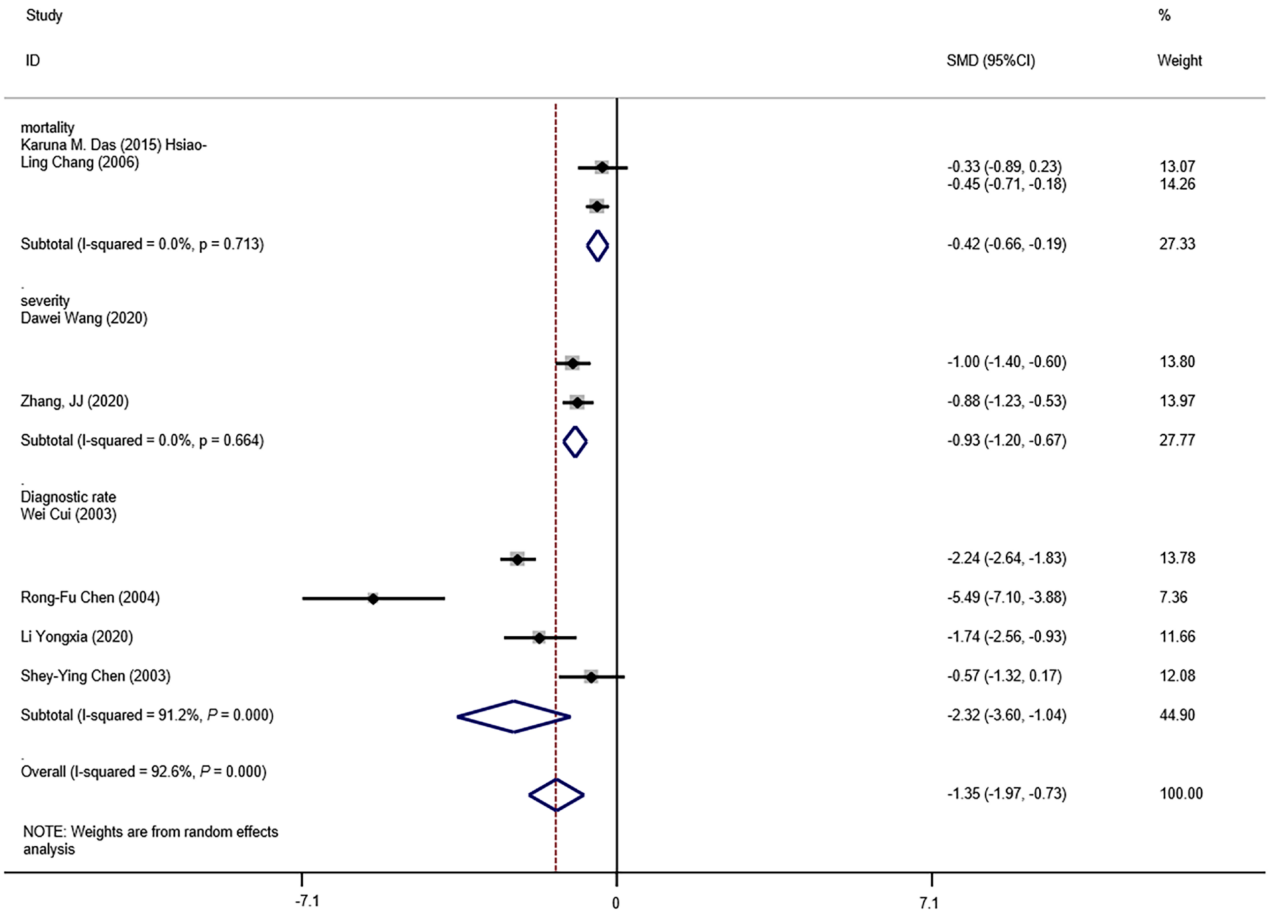

Figure 3 Forest plot: overall identification of lymphocyte count in patients with coronaviral pneumonia, subgroup analysis stratified by prognosis. *P* = 0.000 means *P* < 0.001. CI: Confidence interval.

COVID-19 patients. Because lymphocyte count reflects disease characteristics, it might potentially help in the evaluation of COVID-19 severity. Usefully, this measure is easily available in laboratory tests.

Lymphocytes are vital cells that maintain immune function and execute the immune response in the body[8]. In the best-case scenario, the cellular immune response rapidly clears CoV with little or no clinical signs of infection. Alternatively, the virus causes a state of immunosuppression, which debilitates and sometimes overwhelms the host's defenses[25]. Currently, no detailed study of the immunological response to SARS-CoV-2 is available. Thus, we must rely on previous studies of other CoVs, especially SARS-CoV and MERS-CoV[26]. Based on other CoVs, SARS-CoV-2 might induce a T-lymphocyte-mediated protective immune response[25]. However, lymphopenia is associated with many types of infections, including coronavirus[14,27]. Studies have shown that lymphopenia is related to cell apoptosis in SARS patients[19,28]. In addition, hospitalized patients infected with SARS-CoV-2 frequently manifest lymphopenia, suggesting that cellular immune responses may be suppressed[10,21]. However, lymphopenia in MERS but not SARS cases could be a result of direct infection of T cells and infection-induced apoptosis[29]. Although the lymphopenia in patients with SARS and those with MERS may have different mechanisms, they forecast similar outcome. Furthermore, lymphocyte count as a biomarker to predicting severity in other non-CoV diseases (such as measles, herpes and vaccinia) is effective [19]. Therefore, the hypothesis which predictive role of lymphopenia in COVID-19 is reasonable, and some recently study also provide some evidence to prove this hypothesis[15]. However, studies of the role of lymphocytes in COVID-19 are rare.

In this study, we designed a meta-analysis to explore the feasibility of using lymphocyte count to predict COVID-19 severity. All of the eight studies included in our meta-analysis involved SARS, MERS or COVID-19, and were prospective or retrospective case–control, cohort or cross-sectional studies (Table 1). Existing research results do not always rank the severity of COVID-19 infections using the same criteria. Even during the earliest part of the Chinese outbreak, the guidelines for COVID-19 diagnosis and treatment issued by the Chinese National Health Commission were

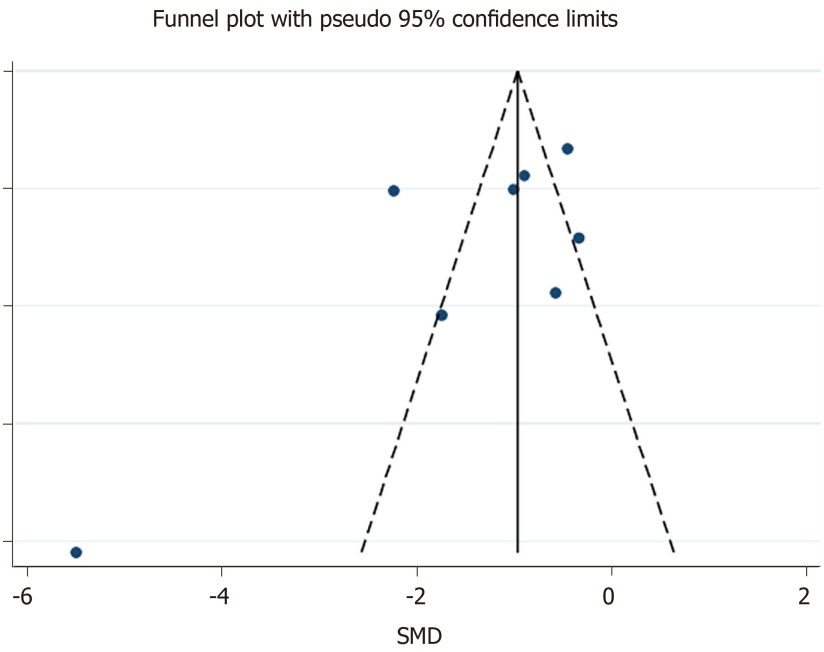

Figure 4 Funnel plot: publication bias of eight studies included.

revised seven times based on clinical experiences[30]. Therefore, we roughly evaluated disease severity using paired concepts: diagnosed *vs* nondiagnosed [19,22-24]; severe *vs* nonsevere[10], and death *vs* survival[13,14].

We found that lymphocyte count was associated with severe CoV infection (Figure 2). Lymphocytes were also associated with CoV mortality, severity and diagnostic rate (Figure 3). Thus, lymphocyte count may represent a simple, rapid, and commonly available diagnostic and severity-prediction tool for CoV infections, including COVID-19. Because we included studies of SARS-CoV-2 infections, our results indicate the value of lymphocyte count for the assessment of COVID-19 severity. A recent related study suggested that lymphocyte percentage might be a reliable indicator of moderate, severe or critical infections, independent of auxiliary indicators[15]. However, this study included few severe (n = 39) and critical (n = 28) cases[15]. In combination, our analysis and the most recent research results suggested that lymphocyte counts potentially reflect COVID-19 severity. Meanwhile, some research has shown that the viral load of SARS-CoV-2 is associated with the lymphocyte count: A study of interleukin (IL)-3 (a cytokine produced by T lymphocytes) discovered that patients with high viral load presented had lower plasma IL-3 levels than low viral load[31]. CD4:CD8 ratio and T regulatory cells significantly decrease in mice with high viral load[32]. Not only T cells, but also B cells and natural killer cells in patients with severe COVID-19 were significantly lower than those in patients with the mild form[33,34]. Lymphopenia in COVID-19 may have several underlying causes, including the destruction of lymphatic organs, the direct attack on lymphocytes by SARS-CoV-2[35], lymphocyte apoptosis due to the continual release of inflammatory cytokines, or, in severe cases, the inhibition of lymphocytes by hyperlactacidemia[36]. It is clear that hyperlacticacidemia- and cytokine-storm-related acute respiratory distress syndrome is the primary presentation of patients with critical COVID-19[30]. Thus, lymphocyte count is an important indicator of severe or critical COVID-19.

For the acute outbreak of COVID-19, the meta-analysis has limitations. We faced a shortage of data sources during the research. Even now, the data on COVID-19 are insufficient. The research results in the previous response to CoVs should be used for reference. The most mainstream of these were SARS and MERS. As other important CoV diseases, they have similarities to COVID-19. From the perspective of the important characteristics of respiratory infections, SARS and MERS could be included in the reference category. The prevention and treatment methods of COVID-19 all often refer to methods of SARS and MERS. A meta-analysis about prevention of person-to-person transmission of COVID-19 also included SARS and MERS coincidentally[37]. Studies have shown that lymphopenia is related to apoptosis in SARS[28]. Lymphopenia is the result of direct T cell infection and infection-induced apoptosis in MERS[29]. Although we think that this conclusion is insufficient, because there are

some treatments (for example: hormone use, antibody titer, *etc*.) that affect the lymphocyte count in the clinic. However, from the current situation of prevalence, spread and treatment, it is of reference value. In studies that were included in the meta-analysis, five referred to lymphocyte counts that were obtained on the day of hospital admission, and two studies did not report clearly the time that lymphocyte counts were obtained. In clinical research, because it is difficult to make a complete review of the course of disease admission, it is generally accepted to choose the day/time of hospital admission as the sampling point[10]. It has been shown that the degree of lymphopenia is steady during the first week[38], and lymphocyte counts progressively decrease and reach their lowest point on day 14 in most cases[38]. Therefore, our analysis did not divide these studies by time of lymphocyte count acquisition. One week of hospital admission could be recognized as a stable interval for lymphocyte counts. However, a recent study showed that lymphocyte count varied at different times of hospitalization[39]. Thus, further study of lymphocyte counts at varied times could increase the predicted efficiency in COVID-19.

CONCLUSION

Our meta-analysis provides information on three simple and common interventions to combat the immediate threat of COVID-19, while new evidence on pharmacological treatments, vaccines, hematology monitoring and other personal protective strategies is being generated[37]. It showed that lymphocyte count may represent a simple, rapid and commonly available laboratory index with which to diagnosis infection and predict the severity of CoV infections, including COVID-19. However, our study was limited by the lack of COVID-19 studies, especially those with homogeneous or uniform standards. Therefore, it was necessary to include SARS and MERS in our study; both of which can lead to coronaviral pneumonia. Thus, these diseases might represent a reference for COVID-19. During the current severe COVID-19 pandemic, we hope to provide a framework for the future predictions of COVID-19 severity. Indeed, if critical infections could be predicted and treated earlier, mortality would be lower. Lymphocyte count is a potential indicator of COVID-19 severity and deserves further examination. However, the high heterogeneity among studies suggests that additional research, including case–control and cohort studies, is required for detailed analyses of the relationship between lymphocyte count and COVID-19 severity.

ARTICLE HIGHLIGHTS

Research background

In December 2019, coronavirus disease 2019 (COVID-19) was reported first in Wuhan, China. COVID-19 is currently a global pandemic.

Research motivation

COVID-19 with high morbidity is a life-threatening disease globally. It is important to develop a rapid, simple clinical method to identify severe COVID-19 cases.

Research objectives

The aim of this study was to assess the suitability of lymphocyte count as a biomarker of COVID-19 severity.

Research methods

We searched five literature databases (PubMed/MEDLINE, Web of Science, Google Scholar, Embase, and Scopus) to identify eligible articles. A meta-analysis was performed to calculate the standard mean difference (SMD) and 95% confidence interval (CI) of lymphocyte counts in coronaviral pneumonia cases.

Research results

Our research integrated eight studies, including 1057 patients. Lymphocyte counts were associated with severe coronavirus (CoV) infection (SMD = 1.35, 95%CI: 1.97 to 0.37, $P < 0.001$, $I^2 = 92.6\%$). In the subgroup analysis stratified by prognosis, lymphocytes were associated with coronavirus infection mortality ($n = 2$, SMD = 0.42, 95%CI: 0.66 to 0.19, $P < 0.001$, $I^2 = 0.0\%$), severity ($n = 2$, SMD = 0.93, 95%CI: 1.20 to

0.67, $P < 0.001$, $I^2 = 0.0\%$), and diagnostic rate ($n = 4$, SMD = 2.32, 95%CI: 3.60 to 1.04, $P < 0.001$, $I^2 = 91.2\%$).

Research conclusions

Lymphocyte count may represent a simple, rapid and commonly available laboratory index with which to diagnosis infection and predict the severity of CoV infections, including COVID-19.

Research perspectives

As a CoV hypotype, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is similar to the CoVs causing severe acute respi-ratory syndrome and Middle East respiratory syndrome. Hence, we performed a systematic review and meta-analysis of the literature to evaluate the diagnostic and prognostic utility of lymphocyte count in patients with viral pneumonia caused by CoV infections. Our aim was to explore the possibility that lymphocyte counts predict COVID-19 severity and provide associated evidence.

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This article also commemorates the Chinese medical staff who gave their lives in the fight against COVID-19.

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LETTER TO THE EDITOR

Can a radioimmunoassay kit be developed for accurate detection of the S protein of severe acute respiratory syndrome coronavirus 2?

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the end of 2019 spread worldwide within only a few months. The screening and timely isolation of infected individuals have been regarded as an effective means of epidemic prevention and control. Therefore, effective screening of infected individuals plays a vital role in epidemic prevention and control. At present, reverse transcription-polymerase chain reaction (RT–PCR) is the main method for the *in vitro* detection of SARS-CoV-2. However, RT–PCR requires certified laboratories, expensive equipment, and trained technicians. Therefore, it is necessary to develop simpler and more convenient methods. Some studies have shown that the PepKAA peptide has a high affinity for the S protein of SARS-CoV-2. The tyrosine in PepKAA is labeled with ^{125}I and used to design a radioimmunoassay kit for the detection of the S protein of SARS-CoV-2, which is of great significance for the early diagnosis of COVID-19.

Key Words: SARS-CoV-2; COVID-19; Spike protein; Detection; Radioimmunoassay kit

Core Tip: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly infectious, and early detection of SARS-CoV-2 is the key factor in preventing another epidemic. Radioimmunoassay (RIA) exhibits high sensitivity and specificity. The detection of the S protein on the surface of SARS-CoV-2 by RIA is expected to be applied for the early diagnosis of coronavirus disease 2019, which may have a considerable impact on the control of the epidemic.

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TO THE EDITOR

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly infectious, and people are generally susceptible to this pathogen. Coronavirus disease 2019 (COVID-19) has caused a global pandemic and has been categorized as a Class B infectious disease in China, and Class A management measures have been implemented[1]. The early laboratory detection of COVID-19 is a key factor for ensuring the early admission, treatment, and timely control of patients to prevent the development of another epidemic[2]. At present, reverse transcription-PCR (RT–PCR) is the main method for the *in vitro* detection of SARS-CoV-2[3]. However, PCR requires certified laboratories, expensive equipment, and trained technicians[4]. The use of a low-cost and simple radioimmunoassay to detect a protein on the surface of SARS-CoV-2 may be of clinical value.

The spike (S) protein is located on the surface of SARS-CoV-2. This protein has a receptor-binding domain (RBD) that can interact with the angiotensin I-converting enzyme 2 (ACE2) receptor in human cells[5]. Souza PFN[6,7] showed that eight antibacterial peptides (*Mo*-CBP$_3$-PepI, *Mo*-CBP$_3$-PepII, *Mo*-CBP$_3$-PepIII, *RcAlb*-PepI, *RcAlb*-PepII, *RcAlb*-PepIII, PepGAT, and PepKAA) can target the S protein of SARS-CoV-2. Of these peptides, *Mo*-CBP$_3$-PepII and PepKAA exhibit the highest affinity. The *Mo*-CBP$_3$-PepII sequence is as follows: Asn-Ile-Gln-Pro-Pro-Cys-Arg-Cys-Cys. The PepKAA sequence is as follows: Lys- Ala- Ala- Asn-Arg-Ile-Lys-Tyr-Phe-Gln. We can label *Mo*-CBP$_3$-PepII or PepKAA sequences using ^{125}I to detect SARS-CoV-2.

Hypothesis

To improve the diagnostic sensitivity of COVID-19, the detection of the S protein of SARS-CoV-2 *via* the RIA method was specifically designed as follows.

The PepKAA sequence is labeled with ^{125}I: This sequence is characterized by: (1) A high affinity for the S protein of SARS-CoV-2; and (2) the existence of a tyrosine within the sequence for easy ^{125}I labeling. This sequence can be easily synthesized *via* solid-phase polypeptide synthesis, requiring only ten amino acids (Lys-Ala-Ala-Asn-Arg-Ile-Lys-Tyr-Phe-Gln). Thus, synthesis can be achieved at a low cost. PepKAA can be labeled *via* the chloramine-T method, which is simple and constitutes a mature method.

Preparation of RIA kit: RIA kits were prepared using following the steps: (1) PepKAA, a peptide targeting the S protein of novel coronavirus, was synthesized by solid-phase peptide synthesis (SPPS); PepKAA was purified by reversed-phase high-performance liquid chromatography (RP-HPLC). After purification, the peptide was analyzed by mass spectrometry; (2) ^{125}I labeling of PepKAA based on the chloramine T method was performed. One hundred and fifty micrograms of PepKAA powder was dissolved in 20 μL DMSO and then added to PB buffer (pH = 7.4) to generate 200 μL of PepKAA solution. After adding 20 μL of chloramine-T solution (5 mg/mL) into a mixed solution of 200 μL PepKAA and 10 μL of Na^{125}I (1.04 mci), the solution was placed in a mixer to react for 50 s at room temperature. Then, 150 μL of sodium metabisulfite (5 mg/mL) was added to terminate the reaction, and ^{125}I-PepKAA was purified using an activated C18 column; (3) preparation of coronavirus-inactivated specimens at six concentrations, including 100 ng/mL, 30 ng/mL, 10 ng/mL, 3 ng/mL, 1 ng/mL, and 0.3 ng/mL; (4) preparation of PEG virus precipitation solution. A 50% PEG solution was first prepared, or solid PEG was directly added to the virus suspension at the required concentration; and (5) the finished solution was packaged, inspected, and stored.

Detection method: Sample collection utilized throat swabs or patient serum. All of the samples were inactivated *via* high temperature before detection, and ^{125}I-labeled PepKAA was added to the samples or standards, which were then incubated in a water bath for 30 min. A PEG virus precipitator was added, and then the mixture was allowed to stand. The supernatant was discarded. Then, a gamma counter was used to measure the radioactive count in each tube (including standard tubes and the

measuring tube). Finally, the virus concentration in the measuring tube was calculated according to the radioactive counts of the standard tubes with different concentrations.

Discussion

Since Yalow and Berson[8] pioneered the development of the first competitive RIA of human insulin in 1959, RIA technology has been applied to a wide variety of fields. RIA has advantages of high sensitivity and specificity[9].

This method exhibits a high specificity. PepKAA was labeled with iodine-125 in this study, and PepKAA can bind specifically to the S protein on the surface of SARS-CoV-2. This method is also highly sensitive. RIA can detect the substance at a level of pg/mL. Other tests cannot achieve this level of sensitivity.

The detection of S protein on the surface of SARS-CoV-2 by RIA is expected to be applied to the early diagnosis of COVID-19, which may have a considerable impact on controlling the epidemic.

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