LETTER

Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus

A series of pneumonia cases caused by 2019 novel coronavirus (2019-nCoV, also named COVID-19) are being reported globally. Based on recent publications,[1–3] the most common symptoms in patients infected by 2019-nCoV were fever and cough. However, the incidence of other clinical features differs in different reports. To address this issue, we collected the data from three reports[1–3] and compared the incidence accordingly. We found that the incidence of leucopenia, fever and diarrhoea in the three studies showed a statistically significant difference (table 1). Among these symptoms, diarrhoea displayed the smallest p-value (p=0.016), suggesting that the criteria for diagnosing diarrhoea may differ in different hospitals. Due to the different criteria, clinicians may underestimate the value of this symptom in clinical practice, and it may affect the preliminary diagnostic accuracy.

Recent studies showed that the spike protein of 2019-nCoV shared the same cell entry receptor ACE2 as SARS-CoV.[4 5] In terms of the pathological importance of ACE2 in modulating intestinal inflammation and diarrhoea,[6] we examined the expression profiles of ACE2 in various human tissues and found that ACE2 was highly expressed in the human small intestine (online supplementary file 1). Intriguingly, the RNA level of ACE2 was quite low in lung tissues from healthy donors.

Given that the distribution of ACE2 may determine the route of 2019-nCoV infection, we next evaluated the expression of ACE2 in different cell populations of the small intestine by analysing the single-cell RNA sequencing (scRNA-Seq) data. Based on the scRNA-Seq data (GSE92332),[7] we analysed 7216 individual cells derived from the small intestine of normal C57BL/6 mice. Using the unsupervised graph-based clustering, we found that the small intestine tissues contained at least eight distinct cell clusters according to their corresponding marker gene expression profiles (figure 1A,B). For instance, the LGR5 gene was highly expressed in the stem cell cluster of the small intestine, and it was significantly reduced in other cell clusters (figure 1B).

We then found that ACE2 was highly expressed in proximal and distal enterocytes (figure 1C,D). Enterocytes are simple columnar epithelial cells located in the inner surface of the small and large intestines. Thus, enterocytes are directly exposed to food and foreign pathogens. Interestingly, when we examined expression profiles of another two virus receptors (ANPEP receptor for HCoV-229E virus and DPP4 receptor for MERS-CoV virus), we found that the RNA levels of these two virus entry receptors were also highly expressed in proximal or distal enterocytes (figure 1E–H), consistent with the expression profile of ACE2.

Currently, the infection routes of 2019-nCoV remain elusive. The distribution of 2019-nCoV entry receptor may determine the path of infection, and the route of infection is essential for understanding the pathogenesis, both of which are vital for infection control in hospitals and society. Based on the current findings, we proposed that (1) the incidence of diarrhoea may be underestimated in previous investigations; (2) ACE2-expressing small intestinal epithelium cells might be more vulnerable to attack by 2019-nCoV.

In this study, we displayed that ACE2 was highly expressed in the small intestine, especially in proximal and distal enterocytes. Consistently, another group has recently reported a similar expression pattern in the human digestive system.[8] Interestingly, other virus receptors like DPP4 displayed similar expression patterns as ACE2 in the small intestine. DPP4 is a known receptor for MERS-CoV through interacting with MERS-CoV spike protein. According to the recent publication, Zhou *et al* reported the DPP4-expressing human intestine cells were highly susceptible to MERS-CoV and supported robust viral replication,[9] suggesting that the human intestinal tract may serve as an alternative infection route for MERS-CoV. In terms of the fact that most of the patients in the outbreak reported a link to a wild animal market, this observation raises an important question about whether this

Table 1 Intergroup comparison between three recent publications

Characteristics	Huang *et al* (41 cases)	Chan *et al* (6 cases)	Chen *et al* (99 cases)	Difference between groups (p value)
Age	49.0 (17.0)	46.2 (28.3)	55.5 (17.7)	–
Sex (female)	11/41 (27%)	3 (50%)	32 (32%)	0.4591
Comorbidity	13/41 (32%)	4 (67%)	50 (51%)	0.0818
Fever	40/41 (98%)	5 (83%)	82 (83%)	0.0345*
Cough	31/41 (76%)	4 (67%)	81 (82%)	0.3829
Diarrhoea	1/38 (3%)	2 (33%)	2 (2%)	0.016†
Shortness of breath/ difficulty in breath	22/40 (55%)	NA	31 (31%)	–
Haemoptysis	2/39 (5%)	NA	NA	–
Sputum production	11/39 (28%)	2 (33%)	NA	–
Myalgia or fatigue	18/41 (44%)	3 (50%)‡	NA	–
Headache	3/38 (8%)	NA	8 (8%)	–
Leucopenia	10/40 (25%)	0 (0%)	9 (9%)	0.0443§
Platelet count	2/40 (5%)	0 (0%)	NA	–

Leucopenia: less white blood cell count (<4*10^9/L); platelet count: <100*10^9/L.
Difference between groups was calculated by Fisher's exact test in R software.
*Huang vs Chan: 0.2414; Chan vs Chen: 1.00; Huang vs Chen: 0.0235.
†Huang vs Chan: 0.044; Chan vs Chen: 0.016; Huang vsChen: 1.00.
‡ Symptom: generalised weakness.
§ Huang vs Chan: 0.3145; Chan vs Chen: 1; Huang vsChen: 0.026.
NA, not available.

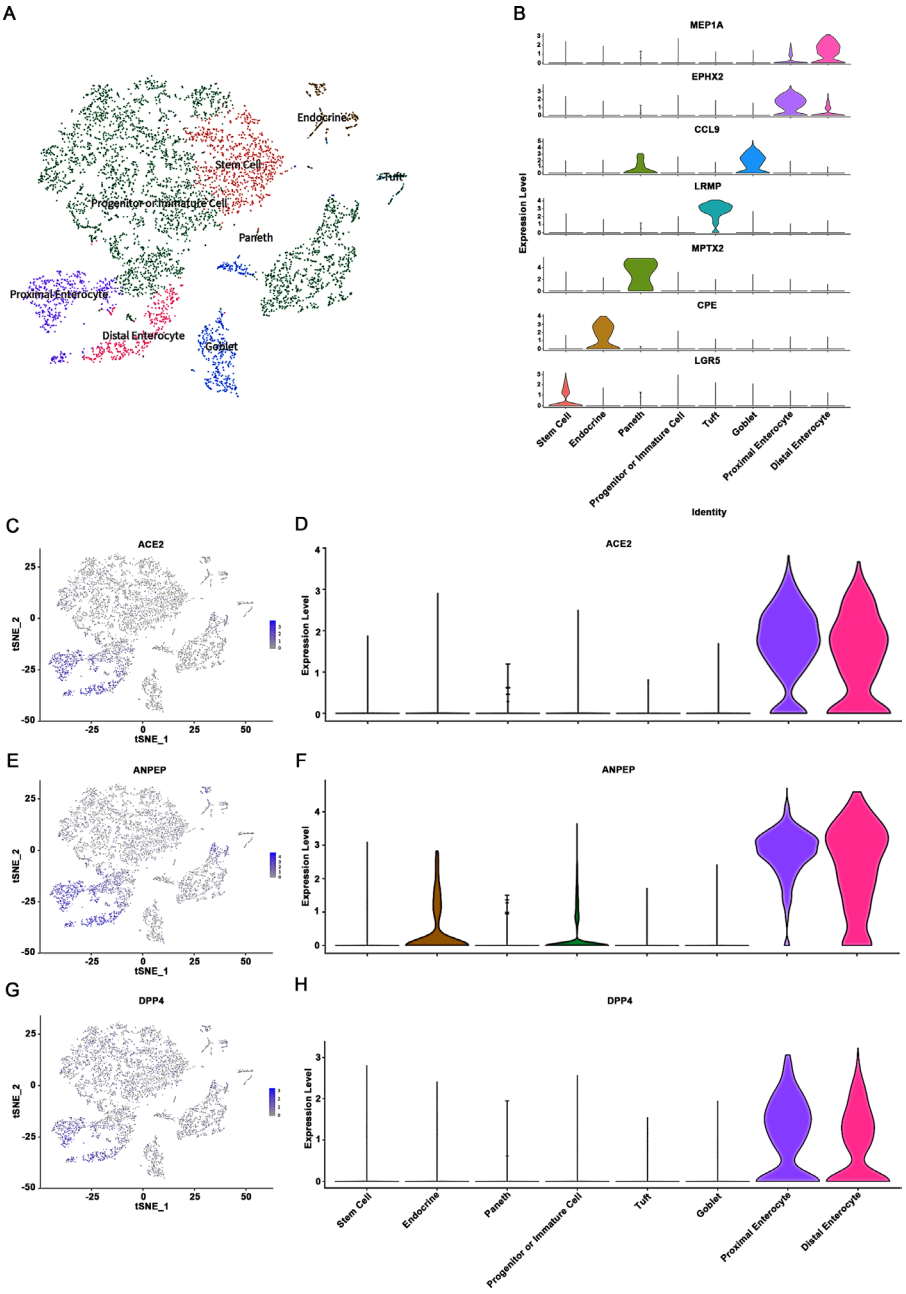


Figure 1 High expression of 2019-nCoV viral receptor ACE2 in enterocytes of small intestine. (A) Analysis of single-cell sequencing data identified eight cell subpopulations within mouse small intestine. (B) Cluster identity was identified by the expression of marker genes and the annotations provided in the UMI-barcode matrix. (C&E&G) Expression profile of three virus receptors ACE2 (2019-nCoV and SARS-CoV), ANPEP (HCoV-229E) and DPP4 (MERS-CoV) in eight subset cell clusters. (D&F&H) Violin plots displayed that ACE2, ANPEP and DPP4 are highly expressed in proximal and distal enterocytes. nCoV, novel coronavirus.

virus is transmitted via contaminated food when the food arrives at the small intestine.

It is known that ACE2 controls intestinal inflammation and diarrhoea. Therefore, mutual interaction between 2019-nCoV and ACE2 might disrupt the function of ACE2 and results in diarrhoea. Here, we found that the incidence of diarrhoea significantly differed in different reports. As 2019-nCoV is highly homologous to SARS-CoV and around 20%–25% of SARS patients have diarrhoea,[10] it is confusing to observe the relatively low incidence (2%–3%) of diarrhoea in two cohorts from hospitals in Wuhan. The underestimation may result from that we still do not have a precise criterion for diarrhoea. The definition of diarrhoea by the WHO is having three or more loose or liquid stools per day or having more stools than a person's health condition. To a certain extent, this criterion is subjective. Besides, we still cannot exclude the effect of the small sample size (n=6) of the Hong Kong cohort, which affects the result of statistical analysis. Emerging evidence shows that 2019-nCoV RNA can be detected in stool samples as SARS. Based on the postulation from the epidemiological features of SARS, which is transmitted through fecal-oral, 2019-nCoV might use the same path for transmission. Thus, future efforts at prevention and control must take into consideration the potential for fecal-mediated spread of this virus.

Taken together, the symptoms of diarrhoea could be underestimated. The information on discharge frequencies and the Bristol stool scale should be carefully collected. When infected patients with diarrhoea visit the gastroenterology department, it may increase the risk of infection of healthcare workers. To reduce healthcare-associated infection, clinicians should be careful when their patients complain of diarrhoea.

Weicheng Liang,[1,2] Zhijie Feng,[3] Shitao Rao,[4] Cuicui Xiao,[1,2] Xingyang Xue,[5] Zexiao Lin,[2,6] Qi Zhang,[1,2] Wei Qi [3]

[1]Cell-gene Therapy Translational Medicine Research Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China
[2]Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou, China
[3]Department of Gastroenterology, The Second Hospital of Hebei Medical University, Hebei Key Laboratory of Gastroenterology, Hebei Institute of Gastroenterology, Shijiazhuang, China
[4]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China
[5]Department of Thoracic Surgery, Affiliated Cancer Hospital & Institute of Guangzhou Medical University, Guangzhou, China
[6]Department of Medical Oncology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Correspondence to Wei Qi, Department of Gastroenterology, The Second Hospital of Hebei Medical University, Hebei Key Laboratory of Gastroenterology, Hebei Institute of Gastroenterology, Shijiazhuang, China; chinaqiwei@yahoo.com and Qi Zhang, Cell-gene Therapy Translational Medicine Research Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; keekee77@126.com

Contributors Study concept was created by WL, QZ and WQ. Data collection was performed by WL, ZF, SR, CX, XX, ZL and WQ. Bioinformatics analysis was conducted by WL, ZF, SR, WQ. Statistical analysis was conducted by ZF and SR. WL, QZ and WQ wrote the manuscript. Funding was obtained by WL and QZ. All authors approved the final version for publication.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Funding This work was supported by grants from the National Natural Science Foundation of China (81870449 and 81670601 to Q.Z., 81902886 to W.L.), Special Fund for Frontier and Key Technology Innovation of Guangdong Province (2015B020226004 to Q.Z.), Key Project Fund of Guangdong Natural Science Foundation (2017A030311034 to Q.Z.), Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (Year 2017 to Q.Z.), National Key Point Research and Invention Program of the Thirteenth (2018ZX10723203 to Q.Z.).

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2020-320832).

WL and ZF are joint first authors.

QZ and WQ are joint senior authors.

To cite Liang W, Feng Z, Rao S, *et al*. *Gut* 2020;**69**:1141–1143.

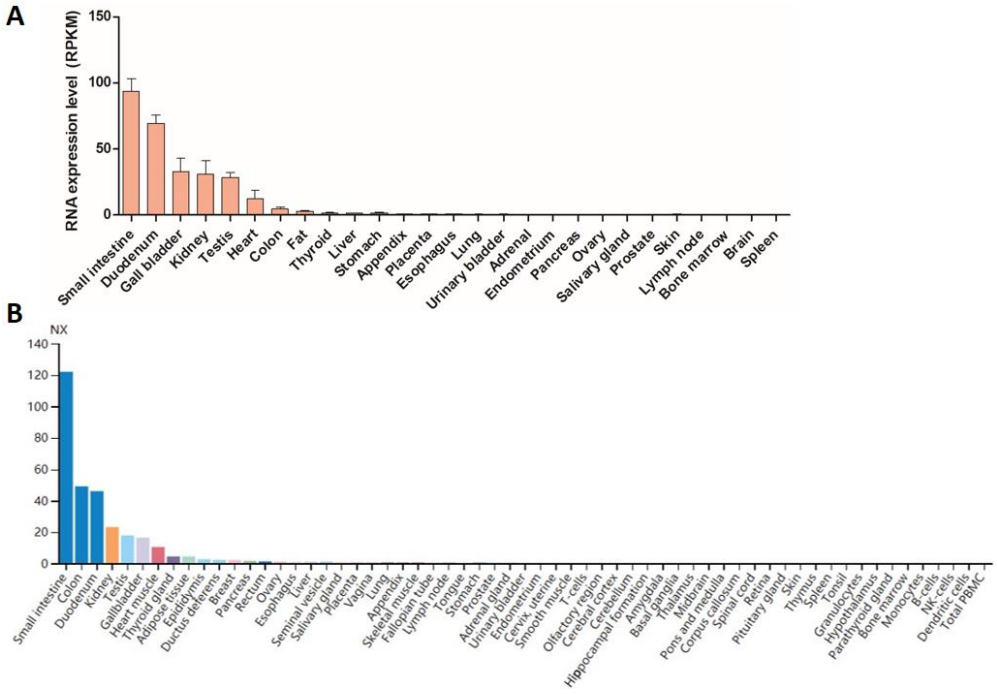
Received 5 February 2020
Revised 12 February 2020
Accepted 13 February 2020
Published Online First 26 February 2020

Gut 2020;**69**:1141–1143. doi:10.1136/gutjnl-2020-320832

ORCID iD
Wei Qi http://orcid.org/0000-0002-0473-092X

REFERENCES

1 Chan JF-W, Yuan S, Kok K-H, *et al*. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;395:514–23.
2 Chen N, Zhou M, Dong X, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020;395:507–13.
3 Huang C, Wang Y, Li X, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497–506.
4 Zhou PYX, Wang X, Hu B, *et al*. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *bioRxiv* 2020.
5 Letko MMV. Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV. *bioRxiv* 2020.
6 Hashimoto T, Perlot T, Rehman A, *et al*. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 2012;487:477–81.
7 Haber AL, Biton M, Rogel N, *et al*. A single-cell survey of the small intestinal epithelium. *Nature* 2017;551:333–9.
8 Zhang HKZ, Gong H, Xu D, *et al*. The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes. *bioRxiv* 2020.
9 Zhou J, Li C, Zhao G, *et al*. Human intestinal tract serves as an alternative infection route for middle East respiratory syndrome coronavirus. *Sci Adv* 2017;3:eaao4966.
10 Wang C, Horby PW, Hayden FG, *et al*. A novel coronavirus outbreak of global health concern. *Lancet* 2020;395:470–3.



Supplementary Figure 1 The expression profiles of ACE2 in normal human tissues. (A) The expression profile of ACE2 from NCBI. (B) The expression profile of ACE2 from The Human Protein Atlas.

Methods

Inter-group difference test

The original information of patients infected by 2019-nCoV infection was retrieved from three recent publications[1-3]. The 2019-nCoV infection was laboratory-confirmed by real-time RT-PCR and next-generation sequencing. Categorical variables were expressed as number with percentage in parenthesis. All the variables were compared by Fisher's exact test for the three studies as one study has a relatively small sample size[1]. Continuous variables were expressed as estimated mean with inter-quartile range (IQR) in parenthesis. A two-sided α of less than 0.05 was considered statistically significant. Statistical analyses were performed using the R software, version 3.5.3.

ACE2 expression profile

The expression profiles of ACE2 from normal human tissues were obtained from public database NCBI (https://www.ncbi.nlm.nih.gov/) with accession number PRJEB4337. The original expression data was collected and then plotted by GraphPad Prism 5.

Single-cell sequencing analysis

The public single-cell RNA-seq sequencing data (GSE92332) was downloaded from the GEO database. The 10X matrix file of GSE92332_atlas_UMIcounts.txt was used for subsequent analysis based on R package Seurat (Version 3.1.2). In order to filter out low-quality cells and low-quality genes, strict parameters, "min.featrue=1000" and "min.cell=20", were used in the function CreateSeuratObject. The data was subsequently log-normalized by the function NormalizeData with the default parameters. The genes with highly variable expression were identified by the function FindVariableGenes. After the data were processed by the function ScaleData, PCA dimensionality reduction was performed utilizing the function RunPCA. Based on the analysis by the function of JackStraw and ScoreJackStraw, the first 15 PCA components were selected for further two-dimensional t-distributed stochastic neighbor embedding (tSNE). The setting of k.param was 30 in the function FindNeighbors, and the setting of the resolution was 1.5 in the function FindClusters. In the downloaded original gene-barcode matrix, Adam L.Haber and his coworkers had annotated the cell identities of each barcode. In our analysis, the cell types were identified by the expression of marker genes and the annotation by the original gene-barcode matrix. Gene expression of different cell types was illustrated by the functions of FeaturePlot and VlnPlot.

Reference:

1 Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020.

2 Chen N ZM, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020.
3 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020.