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laboratory findings, and CT features such as parenchymal involvement and disease progression, both assessed according to the classification by Bernheim and colleagues[4] (appendix). However, lymphadenopathies at admission were significantly more frequent in patients with a crazy paving pattern on CT than in those without (33 [31%] of 106 *vs* 43 [14%] of 304, p<0·001) and in patients who died during hospitalisation than in those who were discharged (37 [27%] of 136 *vs* 39 [14%] of 274, p=0·001; appendix).

Although invasive microbiological samples were not available for our patients (so we cannot exclude bacterial or fungal coinfections), our lymphadenopathy prevalence was lower than that reported by Valette and colleagues[1] but three times higher than estimates for other populations.[2,3,5] We therefore agree in defining lymphadenopathy as a "not-atypical" feature of COVID-19. Furthermore, our data suggest that lymphadenopathy may be considered a predictor of a worse outcome. The pathophysiological meaning of this finding in relation to host response to virus infection and the possibility to use this information in the clinical management of patients with COVID-19 remain to be investigated.

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Pooling of samples for testing for SARS-CoV-2 in asymptomatic people

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The ongoing coronavirus disease 2019 (COVID-19) pandemic is a substantial challenge for health-care systems and their infrastructure. RT-PCR-based diagnostic confirmation of infected individuals is crucial to contain viral spread because infection can be asymptomatic despite high viral loads. Sufficient molecular diagnostic capacity is important for public health interventions such as case detection and isolation, including for health-care professionals.[1]

Protocols for RNA RT-PCR testing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became available early in the pandemic, yet the infrastructure of testing laboratories is stretched and in some areas it is overwhelmed.[2] We propose a testing strategy that is easy to implement and can expand the capacity of the available laboratory infrastructure and test kits when large numbers of asymptomatic people need to be screened. We introduced the pooling of samples before RT-PCR amplification, and only in the case of positive pool test results is work-up of individual samples initiated, thus potentially substantially reducing the number of tests needed.

Viral load during symptomatic infection with SARS-CoV-2 was investigated by Zou and colleagues.[3] To analyse the effect of pooling samples on the sensitivity of RT-PCR, we compared cycle threshold (Ct) values of pools that tested positive with Ct values of individual samples that tested positive.

We isolated RNA from eSwabs (Copan Italia, Brescia, Italy) using the NucliSens easy MAG Instrument (bioMeriéux Deutschland, Nürtingen, Germany) following the manufacturers' instructions. PCR amplification used the RealStar SARS-CoV-2 RT-PCR Kit 1.0 RUO (Altona Diagnostics, Hamburg, Germany) on a Light Cycler 480 II Real-Time PCR Instrument (Roche Diagnostics Deutschland, Mannheim, Germany) according to the manufacturers' instructions.

Our results show that over a range of pool sizes, from four to 30 samples per pool, Ct values of positive pools were between 22 and 29 for the envelope protein gene (E-gene) assay and between 21 and 29 for the spike protein gene (S-gene) assay. Ct values were lower in retested positive individual samples (figure A, B). The Ct values for both E-gene and S-gene assays in pools and individual positive samples were below 30 and easily categorised as positive. Ct value differences between pooled tests and individual positive samples ($Ct_{pool}-Ct_{positive\ sample}$) were in the range of up to five. Even if Ct values of single samples were up to 34, positive pools could still be confidently identified (figure C, D). Sub-pools can further optimise resource use when infection prevalence is low. Generating a pool of 30 samples from three sub-pools of ten samples can reduce retestings.

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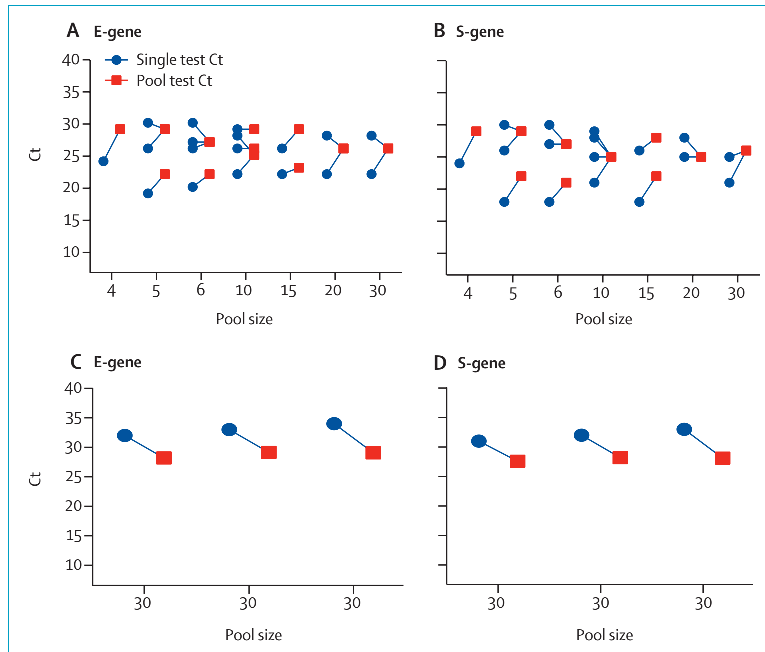

Figure: **Ct values of single versus pooled samples**
Absolute Ct values of positive pools (13 of 164 tested pools) in relation to pool size and corresponding Ct values of individual positive samples for the E-gene assay (A) and the S-gene assay (B). Absolute Ct values were below 30 for all pool sizes. Three positive individual samples with Ct values greater than 30 were spiked into negative pools of 30 samples and tested with E-gene (C) and S-gene (D) assays. We hypothesise that the lower Ct values of pools than of single samples were because of the carrier effect of the higher RNA content in pools. Connecting lines show positive single samples and their corresponding pools. Ct=cycle threshold. E-gene=envelope protein gene. S-gene=spike protein gene.

If the large pool is positive, the three sub-pools are reanalysed, and then the individual samples of the positive sub-pool. In our analyses during March 13–21, 2020, testing of 1191 samples required only 267 tests to detect 23 positive individuals (prevalence 1·93%). The rate of positive tests was 4·24% in our institution during this period.

See Online for appendix

These data suggest that pooling of up to 30 samples per pool can increase test capacity with existing equipment and test kits and detects positive samples with sufficient diagnostic accuracy. We must mention that borderline positive single samples might escape detection in large pools. We see these samples typically in convalescent patients 14–21 days after symptomatic infection. The pool size can accommodate different infection scenarios and be optimised according to infrastructure constraints.

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Challenges and issues of SARS-CoV-2 pool testing

We read with interest Stefan Lohse and colleagues' Correspondence about sample pooling for testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in asymptomatic people.[1] Some of the findings Lohse and colleagues report do not seem to be consistent with other research results[2,3] nor our experiences.

In panels C and D of the figure in Lohse and colleagues' letter,[1] which show the three pooled samples, there is one positive sample in 30 negative samples in each pool, and the pooled samples show lower Ct values than do single samples, which suggests the RNA concentration increased after pooling. Considering that concentrations of RNA had been reduced to 1/31 in the pooled specimens, the Ct values were expected to increase by five compared with single samples. However, in figures C and D, the actual Ct values of the pooled specimens were approximately six values lower than expected, corresponding to a 60-fold increase in RNA concentration.[4] By contrast, we found that when testing pooling of 50 nasopharyngeal and oropharyngeal samples, Ct values (*RdRp* gene) increased with pool size (appendix).

Lohse and colleagues attribute the decreased Ct values to the carrier effect from a higher RNA content in the pool; however, we did not observe a similar phenomenon in 600 tests. Lohse and colleagues did not describe clearly whether the experiment was done with media pooling or swab pooling in a single tube. To our knowledge, the NucliSens easyMAG instrument does not use carrier RNA or DNA for extraction, and there was no evidence to support the carrier phenomenon in the Correspondence.

During our experiments, we observed a few instances wherein the Ct value decreased despite an increased pool size. However, the changes in Ct value