The tremendous ethical and methodological flaws in the Raoult clinical trial: analysis, by Olivier Berruyer

We provide you today with a scientific analysis of the trial on chloroquine conducted by Raoult, Gautret & al. , and which was widely covered by the press two weeks ago, something which has triggered the current controversy.

This is the translation of this article, originally in French. Our apologies for the possible typos…

I. Outline of the article

We will show in this article that the ethical and methodological flaws of the Raoult/Gautret trial made it impossible to interpret its results.

Outline :

II. The Philippe Gautret/ Didier Raoult trial dating from March 2020

2-1 « Hydroxychloroquine plus Azithromycin as a Treatment for covid-19: Results of a Non-Randomized Open-Label Clinical Trial »

2-2 The specifications of the trial

2-3 Criteria for judging the result

2-4 Demographics

2-5 Weird dates

2-1 « Hydroxychloroquine plus Azithromycin as a Treatment for Covid-19: Results of a Non-Randomized Open-Label Clinical Trial »

This trial was published by the IHU of Marseille on its website on March 17 (source, archive, pdf archive) and in the International Journal of Antimicrobial Agents on March 20 (source, archive, pdf archive):

Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial

Philippe Gautret[a,b$], Jean-Christophe Lagier[a,c$], Philippe Parola[a,b], Van Thuan Hoang[a,b,d], Line Meddeb[a], Morgane Mailhe[a], Barbara Doudier[a], Johan Courjon[e,f,g], Valérie Giordanengo[h], Vera Esteves Vieira[a], Hervé Tissot Dupont[a,c], Stéphane Honoré[i,j], Philippe Colson[a,c], Eric Chabrière[a,c], Bernard La Scola[a,c], Jean-Marc Rolain[a,c], Philippe Brouqui[a,c], Didier Raoult[a,c*].

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Traditionally at the IHU (but not only there, certainly), we find a truckload of 18 signatories: Philippe Gautret, Jean-Christophe Lagier, Philippe Parola, Van Thuan Hoang, Line Meddeb, Morgane Mailhe, Barbara Doudier, Johan Courjon, Valérie Giordanengo, Vera Esteves Vieira, Hervé Tissot Dupont, Stéphane Honoré, Philippe Colson, Éric Chabrière, Bernard La Scola, Jean-Marc Rolain, Philippe Brouqui, Didier Raoult.

With so many brains at work, you'd think we'd be dealing with a pretty damn good study…

In addition, let's mention that Raoult presented these results to his students on March 16 on Youtube (source, at 14'31):

2-2 The specifications of the trial

This trial is open-ended and non-randomized : patients know what they are being given, and the distribution was not randomized ; all this greatly reduces the robustness of the trial – but it is still potentially interesting – if we don't jump to conclusions.

Setting

This ongoing study is coordinated by The Méditerranée Infection University Hospital Institute in Marseille. Patients who were proposed a treatment with hydroxychloroquine were recruited and managed in Marseille centre. Controls without hydroxychloroquine treatment were recruited in Marseille, Nice, Avignon and Briançon centers, all located in South France.

So there are going to be 2 groups: one with chloroquine and one without chloroquine.

The group being given chloroquine is at the IHU, the one without it is divided between the IHU, Nice, Avignon and Briançon.

First problem: patients of the control group are scattered in 3 other centres, probably overwhelmed centres too. The big problem is that this multi-centre distribution is carried out **without a distribution of the contaminated patients within each centre**. Marseille is practically the only chloroquine treatment centre where almost 100% of patients are treated, and all the other centres only have control patients.

It is thus impossible to ensure that the protocol is properly followed : the patients could, for example, receive less good care, or simply medical care that is different from what was planned.

Right then, so who are these patients, what are the criteria for joining the study?

Patients

Hospitalized patients with confirmed COVID-19 were included in this study if they fulfilled two primary criteria: i) age >12 years; ii) PCR documented SARS-CoV-2 carriage in nasopharyngeal sample at admission whatever their clinical status.

The investigators therefore decided that 2 criteria had to be met:

- be over **12 years old**
- have som virus at the back of the nose

Health condition is not a criterion :

Clinical classification

Patients were grouped into three categories: asymptomatic, upper respiratory tract infection (URTI) when presenting with rhinitis, pharyngitis, or isolated low-grade fever and myalgia, and lower respiratory tract infections (LRTI) when presenting with symptoms of pneumonia or bronchitis.

So here we are, with three groups of patients :

1. asymptomatic : no clinical signs;
2. « URTI » : who suffer from rhinitis, pharyngitis, or moderate fever and muscle pain;
3. « LRTI » : who suffer from pneumonia or bronchitis.

So there is a gradation of seriousness, even though apparently URTIs can be hospitalized and LRTIs not.

Some patients have been excluded: those with particular pathologies (eye or heart problems) or pregnant women :

Patients were excluded if they had a known allergy to hydroxychloroquine or chloroquine or had another known contraindication to treatment with the study drug, including retinopathy, G6PD deficiency and QT prolongation. Breastfeeding and pregnant patients were excluded

On the other hand, those who were excluded and those who refused treatment were placed in the control group :

Patients who refused the treatment or had an exclusion criteria, served as controls in Marseille centre. Patients in other centers did not receive hydroxychloroquine and served as controls.

New problem: the control group has special characteristics that can alter the efficacy of the treatment for them.

A quick perusal of the article suggests that the trial is over – just look at its title:

Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial

But in fact no, these are actually preliminary results after six days of treatment:

> Clinical follow-up and occurrence of side-effects will be described in a further paper at the end of the trial.

We are led to understand that the trial is to last 14 days

> *Procedure*
>
> Patients were seen at baseline for enrolment, initial data collection and treatment at day-0, and again for daily follow-up during 14 days. Each day, patients received a standardized clinical examination and when possible, a nasopharyngeal sample was collected. All clinical data were collected using standardized questionnaires.

2-3 Criteria for judging the result

When the sponsor plans a clinical trial, the objective and purpose of the trial should be clearly defined.

To do so, for this purpose, **a main criterion** is defined as a » Primary endpoint » corresponding to the main outcome of the study on which the efficacy of the treatment can be concluded.

Generally speaking, trial sponsors also add additional subcriteria. However, only the primary endpoint, if statistically significant, can lead to a conclusion, and this is never the case for the secondary endpoints taken independently. Thus, the trap sometimes suggested by some authors is to conclude on the secondary criteria when the result of the main criterion is not significant. This is not proper and is a significant analytical error, because when the primary endpoint is negative, one should no longer be able to conclude anything from the study based on the secondary endpoints that have been fulfilled. Thus, the role of the secondary endpoints in a study will simply be to supplement the message of the primary endpoint.

Here's the one from the Raoult & Gautret essay:

> *Outcome*
>
> The primary endpoint was virological clearance at day-6 post-inclusion. Secondary outcomes were virological clearance overtime during the study period, clinical follow-up (body temperature, respiratory rate, long of stay at hospital and mortality, and occurrence of side-effects.

With this trial, the primary objective is to **measure the clearance of the virus (in the back of the nose) on day 6 after inclusion.**

The sub criteria will therefore be :

- elimination of the virus (in the back of the nose) on day 14;
- improved clinical outcome: body temperature, respiratory rate, length of hospital stay and **mortality**;
- the occurrence of side effects.

4-4 Demography

All the parameters having been determined, the groups of patients still need to be constituted. To do so, statistics are used to define their size:

> *Statistics*
>
> Assuming a 50% efficacy of hydroxychloroquine in reducing the viral load at day 7, a 85% power, a type I error rate of 5% and 10% loss to follow-up, we calculated that a total of 48 COVID-19 patients (ie, 24 cases in the hydroxychloroquine group and 24 in the control group) would be required for the analysis (Fleiss with CC). Statistical differences were evaluated by Pearson's chi-square or Fisher's exact tests as categorical variables, as appropriate. Means of quantitative data were compared using Student's t-test. Analyses were performed in Stata version 14.2.

The team therefore indicated that their statistical analysis had shown that they would need to round up 48 patients, treating 24 and keeping 24 in a control group.

Problem: Dominique Costagliola, Member of the Academy of Sciences, Vice-Dean Research Delegate of the Faculty of Medicine, Sorbonne University, Deputy Director of the Pierre Louis Institute of Epidemiology and Public Health, at the Sorbonne University, and a specialist in trials, has recalculated the figures and is unable to find the same result. (source)

> *Demographics and clinical presentation*
>
> We enrolled 36 out of 42 patients meeting the inclusion criteria in this study that had at least six days of follow-up at the time of the present analysis. A total of 26 patients received hydroxychloroquine and 16 were control patients.

And thus, the IHU included 42 patients « who met the conditions for inclusion » in this study and divided them into two groups:

- **26 were treated** with hydroxy-chloroquine;
- **16 were the control group**, and were not treated with hydroxychloroquine.

Problem: the control group is significantly smaller (by one third) in size than the 24 from the team's own statistical analysis.

Another problem: the trial is not « randomized ». Patients are not randomly selected to be in the control group or the treatment group. The investigators chose who they were going to treat with hydroxychloroquine (HCQ) (here, the repartition is in fact geographical, and depends on the treatment centre), but, above all, also treated with antibiotic, which represents a very strong bias.

Finally, you will note that they report that they have integrated « 36 of the 42 selected patients ». But this is false; they have integrated all 42 patients! Let's consider what happened.

2-5 No conflicts of interests?

As far as we can tell, the authors haven't mentioned anything concerning eventual conflicts of interest (source) :

N/A = Non available, not to be found

And this is really a pity as this trial is a **IHU Marseille** study

Concerning Chloroquine which is in fact produced by **Sanofi Laboratory**

And Sanofi is one of IHU Marseille's partner(sources there and here)

MÉDITERRANÉE INFECTION

PARTENAIRES

Accueil / L'institut / Partenaires

En plus du financement de l'État, obtenu suite à l'appel d'offre du grand emprunt, ce projet est basé sur des crédits multiples, pour la plupart des partenaires institutionnels, dont l'Assistance Publique (AP-HM), Aix-Marseille Université, l'Institut de Recherche pour le Développement (IRD, dont le siège est à Marseille qui comporte 3 unités mixtes), l'INSERM, le CNRS, l'École des Hautes Études en Santé Publique (EHESP) et l'Établissement Français du Sang (EFS), le Service de Santé des Armées (SSA), les CHU de Montpellier, Nîmes, Nice, ainsi que les Universités de Montpellier 1 et Montpellier 2, et de Nice-Sophia-Antipolis.

Des partenaires privés font également partie du projet parmi les plus **grandes industries nationales** dans le domaine des maladies infectieuses et de la microbiologie (Institut Mérieux, Sanofi Aventis, Cerba European Lab, IRT Lyon, Qiagen) mais aussi des **entreprises locorégionales** (Galderma, Consortium MediHandtrace, I2a) ainsi que le Crédit Coopératif, qui soutenait déjà la Fondation Infectiopôle Sud, et la Caisse d'Epargne.

Here are the partners of IHU-IM

Institut Hospitalo-Universitaire

MÉDITERRANÉE INFECTION

Valorisation/support de nos recherches : nature Clinical Infectious Diseases EMERGING INFECTIOUS DISEASES The ISME Journal ANNUAL REVIEWS CONNECT WITH OUR EXPERTS

- 5 publications représentatives : Desnues *et al.*, 2008 *Nature* ; Fancello *et al.*, 2013 *ISME Journal*, Popgeorgiev *et al.*, 2013 *Journal of Infectious Diseases*, Appelt *et al.*, 2014 *Applied and Environmental Microbiology*, Rascovan *et al.*, 2016 *Clinical Infectious Diseases*.

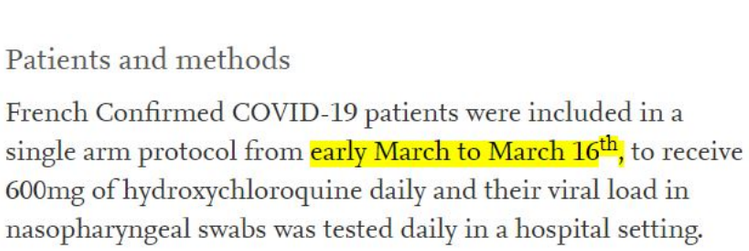
- Nos travaux sont soutenus par le **Conseil Européen de la Recherche** (Starting Grant 242729), L'**Agence Nationale de la Recherche**(ANR-13-JSV6-0004, programme JCJC), la **fondation TOTAL, SANOFI-PASTEUR** et les crédits récurrents du CNRS

Be aware that **Sanofi Aventis is one of the funder that finances the Institute,** which means that Raoult often meets with them (this is the 3rd largest pharmaceutical laboratory in the world. One just has to keep in mind that there is a link there.

2-6 Weird dates

We would like to draw your attention on the problem concerning the dates for this test.

This is what the published article indicates (source, archive) :



Patients and methods

French Confirmed COVID-19 patients were included in a single arm protocol from early March to March 16th, to receive 600mg of hydroxychloroquine daily and their viral load in nasopharyngeal swabs was tested daily in a hospital setting.

Patients were therefore included in « a single-arm protocol **from early March to March 16th.** »

First of all, the characteristic precision of the Marseille IHU on the « beginning of March » is to be noted. They therefore do not seem to want to indicate what the first calendar day of the trial is.

This being said, they indicate an ending date: March 16th.

But the trial is a 14-day trial, with a primary assessment criterion on day 6 (or day 7, depending on how you count the day of inclusion, the team speaks of D0 as we have seen, D for day).

So there are only 2 options: either March16 th is D6, or it is D14.

2-6-1 Scenario D14

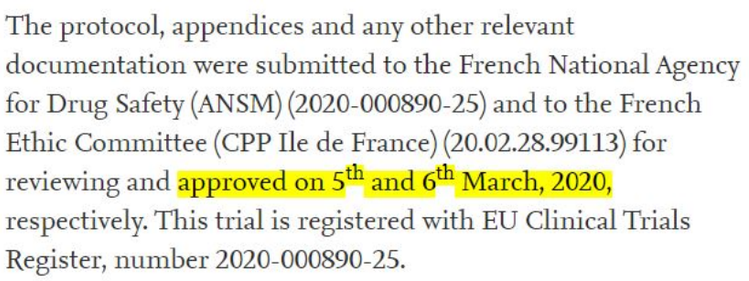
This hypothesis is supported by the sentence mentioning a single-arm protocol from early March to March16. » This trial is not supposed to have two protocols.

Therefore, assuming March 16th is D14, this would mean that inclusion day D0 would be March 2nd, and patients would have been treated from March 3rd to March 16th, and therefore D6 would be March 8th and the trial would have ended on March 17th.

Furthermore, since Didier Raoult presented the D6 findings on March 16th, that would have allowed the team one week to analyze the results and write the article.

But in fact, this doesn't seem feasible. First of all, if the trial ended that day, why « urgently » publish an evaluation at D6 on that same March 16th ?

And more important, we are told that the trial was not approved by the authorities until March 5th and 6 th :



The protocol, appendices and any other relevant documentation were submitted to the French National Agency for Drug Safety (ANSM) (2020-000890-25) and to the French Ethic Committee (CPP Ile de France) (20.02.28.99113) for reviewing and approved on 5th and 6th March, 2020, respectively. This trial is registered with EU Clinical Trials Register, number 2020-000890-25.

It is obviously illegal to carry out a clinical trial without the agreement of the authorities, and this is a criminal offence:

Article L.1126-5 of the Public Health Code

« Conducting, or having conducted, research involving a human being […] without having obtained the positive approval of a committee for the protection of persons shall be liable to one year's imprisonment and a fine of 15,000 euros ».

Which means that D0 can only be, at best, on March 6th or 7th, and therefore D6 can only be Thursday March 12th or Friday 13th .

Since assumption D14 seems to be invalidated, then the following one is the correct one.

2-6-1 Scenario D6

We then have to admit that the wording of the protocol is unfortunate, so March 16th is D6, which would mean that D0, inclusion day would be March 10th, and that the patients would have been treated from March 11th to March 24th, and then the trial ended on March 25th.

But when you look at the publication:

> Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial
>
> Philippe Gautret [a, b, $], Jean-Christophe Lagier [a, c, $], Philippe Parola [a, b], Van Thuan Hoang [a, b, d], Line Meddeb [a], Morgane Mailhe [a], Barbara Doudier [a], Johan Courjon [e, f, g], Valérie Giordanengo [h], Vera Esteves Vieira [a], Hervé Tissot Dupont [a, c], Stéphane Honoré [i, j], Philippe Colson [a, c], Eric Chabrière [a, c], Bernard La Scola [a, c], Jean-Marc Rolain [a, c], Philippe Brouqui [a, c], Didier Raoult [a, c]
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> [d] Thai Binh University of Medicine and Pharmacy, Thai Binh, Viet Nam
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> [f] Université Côte d'Azur, Nice, France
> [g] U1065, Centre Méditerranéen de Médecine Moléculaire, C3M, Virulence Microbienne et Signalisation Inflammatoire, INSERM, Nice, France
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>
> Received 16 March 2020, Accepted 17 March 2020, Available online 20 March 2020.

So we can see that it means that on Monday, March 16th, it would have been necessary to :

- collect the tests from all 30 patients;
- perform all 30 tests;
- report and synthesize the results in Marseille;
- carry out the complete study and the graphs;
- have it re-read and signed by the 18 people;
- get the slides ready for the power point presented by Didier Raoult on March 16th ;

- Send the article to the International Journal of Antimicrobial Agents (IJAA) for review.

So on March 17th, the IJAA found a proofreader (maybe two), who proofread this article and validated it **within the same day** – that's really fast. Too fast, it seems…

As can be seen, this hypothesis, although legal, raises serious problems of credibility.

Problem: It is very difficult to establish the calendar dates D0, D6 and D14 for this clinical trial.

III. Result of the trial and discussion

3-1 Strange lost people !

3-2 The 36 patients (remaining)

3-3 Hydroxychloroquine results

3-4 Detailed patient data

3-5 The problem of test reliability

3-6 Analysis of the results for hydroxychloroquine

3-7 Results for hydroxychloroquine plus azithromycin

3-8 Viral carriage

3-9 Let's put some seriousness into this trial…

3-10 One last big problem

As we previously mentioned, understanding a clinical trial is not very complicated… Let's move on to the analysis of the results.

3-1 Strange lost people !

Clinical trials for drug development are frequently carried out on thousands of patients over trimesters or even years. It may therefore happen that some people leave the trial without informing anyone (relocation, weariness, etc.). This is what is called « **lost people** » : they were there at the beginning, but are no longer there at the end, without the reason necessarily being known.

And, you won't believe this, but out of the 26 prople treated with chloroquine, **the authors indicate that there were… 6 lost people within 6 days!**

Six hydroxychloroquine-treated patients were lost in follow-up during the survey because of early cessation of treatment. Reasons are

So all those who have « **stopped the treatment early** » are called « lost« . And the reasons why they did so are very interesting:

one patient decided to leave the hospital on day3 post-inclusion and was PCR-negative on days1-2; finally, one patient stopped the treatment on day3 post-inclusion because of nausea and was PCR-positive on days1-2-3.

The first of those decided to leave the hospital on day 3, and by days 1 and 2 he had no virus left in his samples. That's called a **healed one**. On the one hand, we can say that it was a success, but on the other hand, the treatment obviously had **nothing** to do with it. One can even wonder if he was even really sick the day the trial started.

The second decided to voluntarily stop the treatment because of nausea on the 3rd day, while he was still infected with the virus. So we can imagine that he must have been extremely nauseated to choose to stop the treatment… So in fact he is not « lost » at all: it is a classical case of treatment **failure, linked to side effects that are too difficult to cope with.**

But let's continue the analysis of our lost to follow up patients:

three patients were transferred to intensive care unit, including one transferred on day2 post-inclusion who was PCR-positive on day1, one transferred on day3 post-inclusion who was PCR-positive on days1-2 and one transferred on day4 post-inclusion who was PCR-positive on day1 and day3;

Here's something different: 3 have simply gone into emergency intensive care ! On days two, three and four. So these are serious **treatment failures**.

For the sixth « lost » patient it's even worse :

one patient died on day3 post inclusion and was PCR-negative on day2;

He died on the third day.

His nose testing revealed that he was not carrying the virus anymore.

So he died with no virus in his nose… (which is frequent, death is often actually due to a bursting of the immune defences. But the virus could also be somewhere else)

So here we are again with another serious chloroquine treatment failure.

And **Raoult's team swept those 5 failures out of the study, and quietly made them look like lost to follow up patients !**

Which in fact means that they gave a patient a new treatment, he died 3 days after that, they just shrugged their shoulders and dropped him from the study as if he had decided to go home. Same for the 3 sent to intensive care and the one with intolerable side effects.

Never have I seen something of the kind in a test report before ! This comes close to the threshold of scientific fraud.

Quite obviously, there's no evidence that chloroquine was involved in any of the five failures. But there's also nothing to say that it wasn't.

Because in fact, in the control group (without chloroquine): no death, **nor any transfer to the intensive care unit:**

None of the control patients was lost in follow-up.

This might have been a piece of luck, it might have been normal. Or maybe it wasn't…

For those of you that are interested, here is a summary of what is now a nugget among clinical trials:

Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial

We enrolled 36 out of 42 patients meeting the inclusion criteria in this study that had at least six days of follow-up at the time of the present analysis. A total of 26 patients received hydroxychloroquine and 16 were control patients. Six hydroxychloroquine-treated patients were lost in follow-up during the survey because of early cessation of treatment. Reasons are as follows: three patients were transferred to intensive care unit, including one transferred on day2 post-inclusion who was PCR-positive on day1, one transferred on day3 post-inclusion who was PCR-positive on days1-2 and one transferred on day4 post-inclusion who was PCR-positive on day1 and day3; one patient died on day3 post inclusion and was PCR-negative on day2; one patient decided to leave the hospital on day3 post-inclusion and was PCR-negative on days1-2; finally, one patient stopped the treatment on day3 post-inclusion because of nausea and was PCR-positive on days1-2-3. The results presented here are therefore those of 36 patients (20 hydroxychloroquine-treated patients and 16 control patients). None of the control patients was lost in follow-up. Basic demographics and clinical status are presented in Table 1.

But let's carry on, because the study, having camouflaged its failures, continues – on the basis of only 20 patients treated with chloroquine and still 16 patients in the control group:

The results presented here are therefore those of 36 patients (20 hydroxychloroquine-treated patients and 16 control patients).

Problem: 20 and 16 are therefore quite far from the statistical need for 24 and 24.

And it is strange to note that, while the number of patients treated with chloroquine was 26 at the beginning and 20 at the end, Raoult publicly speaks of « 24 treated patients » (sources here, here and here) :

Menée sur seulement 24 malades et suivant une méthodologie jugée contestable par certains, la première étude du professeur Raoult avait été vivement critiquée. Le spécialiste des maladies infectieuses a donc

de la chloroquine. Ce lundi 16 mars, dans un exposé vidéo rencontrant un succès "viral" sur les réseaux sociaux, le professeur expose en effet les résultats positifs de ses essais cliniques : sur 24 patients atteints du coronavirus, les trois quarts étaient guéris en six jours après avoir reçu de la chloroquine.

La chloroquine, un remède miracle contre le coronavirus ? Spécialiste des maladies infectieuses à Marseille, le professeur Didier Raoult le proclame partout, après avoir testé ce médicament antipaludique sur 24 patients. Selon lui, les trois quarts étaient guéris au bout de six jours.

3-2 The 36 (remaining) patients

Let's then discover these 36 patients ho are still present in the trial on the 6th day:

Supplementary Table 1.

Patient	Age (years)	Sex	Clinical status	Time between onset of symptoms and inclusion (days)	Hydroxychloroquine treatment	Hydroxychloroquine serum concentration µg/ml (day of dosage)	Azithromycin treatment	D0	D1	D2	D3	D4	D5	D6
1	10	M	Asymptomatic	-	No	-	No	31	NEG	NEG	NEG	NEG	NEG	NEG
2	12	F	Asymptomatic	-	No	-	No	26	ND	33	34	NEG	34	NEG
3	14	F	Asymptomatic	-	No	-	No	26	31	23	22	27	NEG	26
4	10	M	Asymptomatic	-	No	-	No	24	NEG	33	33	NEG	NEG	32
5	20	M	URTI	4	No	-	No	24	24	24	27	NEG	31	29
6	65	F	URTI	2	No	-	No	POS	ND	POS	ND	POS	ND	POS
7	46	M	URTI	Unknown	No	-	No	28	ND	ND	ND	26	ND	30
8	69	M	LRTI	2	No	-	No	POS	ND	POS	ND	POS	POS	POS
9	62	F	LRTI	10	No	-	No	POS	ND	POS	ND	POS	ND	POS
10	66	F	URTI	0	No	-	No	POS	ND	POS	ND	ND	ND	POS
11	75	F	URTI	3	No	-	No	POS	ND	POS	ND	POS	ND	ND
12	23	F	URTI	5	No	-	No	ND	ND	POS	ND	POS	ND	ND
13	45	F	URTI	Unknown	No	-	No	POS	ND	POS	ND	POS	ND	POS
14	16	M	URTI	2	No	-	No	POS	ND	POS	ND	ND	POS	ND
15	42	F	URTI	5	No	-	No	ND	ND	ND	POS	ND	POS	ND
16	23	F	URTI	6	No	-	No	POS	ND	ND	ND	ND	POS	ND
17	44	F	URTI	6	Yes	0.519 (D6)	No	30	ND	29	26	32	26	31
18	54	M	Asymptomatic	-	Yes	0.462 (D6)	No	29	NEG	NEG	NEG	NEG	NEG	NEG
19	25	M	URTI	3	Yes	0.419 (D6)	No	23	25	28	25	NEG	NEG	NEG
20	59	F	Asymptomatic	-	Yes	0.288 (D4)	No	30	NEG	NEG	NEG	NEG	NEG	NEG
21	49	F	URTI	1	Yes	0.621 (D6)	No	34	27	19	16	34	24	22
22	24	F	URTI	10	Yes	0.723 (D6)	No	28	NEG	32	34	NEG	NEG	NEG
23	81	F	LRTI	2	Yes	0.591 (D6)	No	22	21	30	NEG	32	28	NEG
24	85	F	LRTI	1	Yes	0.619 (D6)	No	17	21	23	21	26	24	24
25	40	M	URTI	3	Yes	0.418 (D6)	No	22	ND	28	21	15	20	17
26	53	M	URTI	5	Yes	0.515 (D6)	No	27	28	32	31	NEG	NEG	NEG
27	63	F	URTI	8	Yes	0.319 (D4)	No	34	NEG	30	NEG	NEG	NEG	NEG
28	42	F	URTI	1	Yes	0.453 (D6)	No	19	16	17	17	19	20	31
29	87	F	URTI	5	Yes	0.557 (D6)	No	25	30	NEG	NEG	NEG	ND	ND
30	33	M	URTI	2	Yes	0.194 (D2)	No	15	23	26	26	NF	32	32
31	53	F	LTRI	7	Yes	1.076 (D6)	Yes	28	31	34	NEG	34	NEG	NEG
32	48	M	URTI	2	Yes	0.57 (D6)	Yes	23	29	29	NEG	NEG	NEG	NEG
33	50	F	LRTI	5	Yes	0.827 (D6)	Yes	30	27	NEG	NEG	NEG	NEG	NEG
34	20	M	URTI	2	Yes	0.381 (D6)	Yes	27	31	29	NEG	NEG	NEG	NEG
35	54	M	LRTI	6	Yes	0.366 (D4)	Yes	24	ND	ND	29	NEG	NEG	NEG
36	60	M	LRTI	4	Yes	0.319 (D4)	Yes	29	31	31	NEG	NEG	NEG	NEG

URTI: upper tract respiratory infection, LRTI: lower tract respiratory infection, POS: positive PCR, NEG: negative PCR (CT value ≥35), ND: PCR not done

Please cite this work as Gautret et al. (2020) Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents – In Press 17 March 2020 – DOI : 10.1016/j.ijantimicag.2020.105949

This quite difficult-to-read chart mainly deals with: their age, sex, clinical status, progress in the disease, whether they were treated with hydroxychloroquine and its concentration in the blood, and their virus load on each of the 6 days.

One detail stands out. Let's remember this:

We enrolled 36 out of 42 patients meeting the inclusion criteria

hese are 36 patients who « meet the inclusion criteria ». Criteria which are simple:

Patients

Hospitalized patients with confirmed COVID-19 were included in this study if they fulfilled two primary criteria: i) age >12 years; ii) PCR documented SARS-CoV-2 carriage in nasopharyngeal sample at admission whatever their clinical status.

here are only two of them :

1. **Be over twelve years old**
2. Having some virus in the nose

Good enough

And then, what do we notice ?

Patient	Age (years)	Sex
1	10	M
2	12	F
3	14	F
4	10	M

Patients 1 and 4 are ten years old : they don't satisfy the admission criterion for the trial (eventhough they did not get chloroquine).

That's it, let's call it game over. Bravo « to the most cited microbiology researcher in France ».

But let's go on.

Let us point out another problem: there are serious differences in the data for the control group if we consider the pre-publication of 20 March on medRxiv (source) and the final, authoritative one on the IJAA journal on ScienceDirect (also 20 March – source) (this was pointed out Leonid Schneider, from a PubPeer alert) :

Hydroxychloroquine and Azithromycin as a treatment of COVID-19: preliminary results of an open-label non-randomized clinical trial

Posted March 20, 2020.

doi: https://doi.org/10.1101/2020.03.16.20037135
Now published in ScienceDirect doi: 10.1016/j.ijantimicag.2020.105949

Supplementary Table 1.

Patient	Age (years)	Sex	Clinical status	Time between onset of symptoms and inclusion (days)	Hydroxychloroquine treatment	Hydroxychloroquine serum concentration µg/ml (day of dosage)	Azithromycin treatment	D0	D1	D2	D3	D4	D5	D6
	10	M	Asymptomatic	-	No	-	No	31	NEG	NEG	NEG	NEG	NEG	NEG
	12	F	Asymptomatic	-	No	-	No	26	ND	33	34	NEG	34	NEG
	14	F	Asymptomatic	-	No	-	No	26	31	23	22	27	NEG	26
	10	M	Asymptomatic	-	No	-	No	24	NEG	33	33	NEG	NEG	32
	20	M	URTI	4	No	-	No	24	24	24	27	NEG	31	29
	65	F	URTI	2	No	-	No	POS	ND	POS	ND	POS	ND	POS
	46	M	URTI	Unknown	No	-	No	28	NF	NF	NF	26	NF	30
	69	M	LRTI	2	No	-	No	POS	POS	POS	POS	POS	POS	POS
	62	F	LRTI	10	No	-	No	POS	ND	POS	ND	POS	ND	POS
	66	F	URTI	0	No	-	No	POS	ND	POS	ND	POS	ND	POS
	75	F	URTI	3	No	-	No	POS	ND	POS	ND	POS	ND	POS
	23	F	URTI	5	No	-	No	POS	ND	POS	ND	POS	ND	POS
	45	F	URTI	Unknown	No	-	No	POS	ND	POS	ND	POS	ND	POS
	16	M	URTI	2	No	-	No	POS	ND	ND	POS	ND	POS	POS
	42	F	URTI	5	No	-	No	ND	POS	ND	POS	ND	POS	POS
	23	F	URTI	6	No	-	No	ND	POS	ND	POS	ND	POS	POS

Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial

Available online 20 March 2020
https://doi.org/10.1016/j.ijantimicag.2020.105949

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Patient	Age (years)	Sex	Clinical status	Time between onset of symptoms and inclusion (days)	Hydroxychloroquine treatment	Hydroxychloroquine serum concentration µg/ml (day of dosage)	Azithromycin treatment	D0	D1	D2	D3	D4	D5	D6
1	10	M	Asymptomatic	-	No	-	No	31	NEG	NEG	NEG	NEG	NEG	NEG
2	12	F	Asymptomatic	-	No	-	No	26	ND	33	34	NEG	34	NEG
3	14	F	Asymptomatic	-	No	-	No	26	31	23	22	27	NEG	26
4	10	M	Asymptomatic	-	No	-	No	24	NEG	33	33	NEG	NEG	32
5	20	M	URTI	4	No	-	No	24	24	24	27	NEG	31	29
6	65	F	URTI	2	No	-	No	POS	ND	POS	ND	POS	ND	POS
7	46	M	URTI	Unknown	No	-	No	28	ND	ND	ND	26	ND	30
8	69	M	LRTI	2	No	-	No	POS	ND	POS	ND	POS	POS	POS
9	62	F	LRTI	10	No	-	No	POS	ND	POS	ND	POS	ND	POS
10	66	F	URTI	0	No	-	No	POS	ND	POS	ND	ND	ND	POS
11	75	F	URTI	3	No	-	No	POS	ND	POS	ND	POS	ND	ND
12	23	F	URTI	5	No	-	No	ND	ND	POS	ND	POS	ND	ND
13	45	F	URTI	Unknown	No	-	No	POS	ND	POS	ND	POS	ND	POS
14	16	M	URTI	2	No	-	No	POS	ND	POS	ND	ND	POS	ND
15	42	F	URTI	5	No	-	No	ND	ND	ND	POS	ND	POS	ND
16	23	F	URTI	6	No	-	No	POS	ND	ND	ND	ND	POS	ND

Problem: Was the collection of test data from the control group centers really reliable?

3-3 Hydroxychloroquine results

The potential efficacy of chloroquine in vitro, when poured on cell cultures that are in test tubes, has been repeatedly cited by investigators:

> Hydroxychloroquine (an analogue of chloroquine) has been demonstrated to have an anti-SARS-CoV activity *in vitro* [12].

It just happens that **the *in vitro* efficacy says nothing about the effictiveness in humans**. And the team knows this very well, since for 10 years they've been trying this against many viruses, with no proven effectiveness in humans as to reducing the viral load – but even with proven efficacy in increasing the viral load of infections such as AIDS, Chikungunya and influenza, as demonstrated in this post.

Here is the result as reported:

> *Effect of hydroxychloroquine on viral load*
>
> The proportion of patients that had negative PCR results in nasopharyngeal samples significantly differed between treated patients and controls at days 3-4-5 and 6 post-inclusion (Table 2). At day6 post-inclusion, 70% of hydroxychloroquine-treated patients were virologicaly cured comparing with 12.5% in the control group (p= 0.001).

After 6 days, « **70% of patients treated with chloroquine » were « cured of the virus », compared with « 12.5% in the control group ».**

Sounds impressive when you put it like that. It sounds very convincing. As long as you don't reviez it, of course.

So, let's review it.

3-4 Detailed data on the patients

But first of all let's have a close look at the patients

The chart provided in the article being all mixed up and not very clear, we have redrawn it, more neatly, classifying the patients in a better way :

Patient	Age	Sexe	Statut clinique	Durée entre 1er symptomes et inclusion	Hydroxy-chloroquine	Hydroxy-chloroquine concentration µg/ml (jour de dosage)	Azithromicine	D0	D1	D2	D3	D4	D5	D6
1	10	M	Asymptomatique	--	Non	--	Non	31	NEG	NEG	NEG	NEG	NEG	NEG
4	10	M	Asymptomatique	--	Non	--	Non	24	NEG	33	33	NEG	NEG	32
2	12	F	Asymptomatique	--	Non	--	Non	26	ND	33	34	NEG	34	NEG
3	14	F	Asymptomatique	--	Non	--	Non	26	31	23	22	27	NEG	26
13	45	F	URTI	Inconnu	Non	--	Non	POS	ND	POS	ND	POS	ND	POS
7	46	M	URTI	Inconnu	Non	--	Non	28	ND	ND	ND	26	ND	30
10	66	F	URTI	0	Non	--	Non	POS	ND	POS	ND	ND	ND	POS
14	16	M	URTI	2	Non	--	Non	POS	ND	POS	ND	ND	POS	ND
6	65	F	URTI	2	Non	--	Non	POS	ND	POS	ND	POS	ND	POS
11	75	F	URTI	3	Non	--	Non	POS	ND	POS	ND	POS	ND	ND
5	20	M	URTI	4	Non	--	Non	24	24	24	27	NEG	31	29
12	23	F	URTI	5	Non	--	Non	ND	ND	POS	ND	POS	ND	ND
15	42	F	URTI	5	Non	--	Non	ND	ND	ND	POS	ND	POS	ND
16	23	F	URTI	6	Non	--	Non	POS	ND	ND	ND	ND	POS	ND
8	69	M	LRTI	2	Non	--	Non	POS	ND	POS	ND	POS	POS	POS
9	62	F	LRTI	10	Non	--	Non	POS	ND	POS	ND	POS	ND	POS
18	54	M	Asymptomatique	--	Oui	0.462 (D6)	Non	29	NEG	NEG	NEG	NEG	NEG	NEG
20	59	F	Asymptomatique	--	Oui	0.288 (D4)	Non	30	NEG	NEG	NEG	NEG	NEG	NEG
28	42	F	URTI	1	Oui	0.453 (D6)	Non	19	16	17	17	19	20	31
21	49	F	URTI	1	Oui	0.621 (D6)	Non	34	27	19	16	34	24	22
30	33	M	URTI	2	Oui	0.194 (D2)	Non	15	23	26	26	ND	32	32
19	25	M	URTI	3	Oui	0.419 (D6)	Non	23	25	28	25	NEG	NEG	NEG
25	40	M	URTI	3	Oui	0.418 (D6)	Non	22	ND	28	21	15	20	17
26	53	M	URTI	5	Oui	0.515 (D6)	Non	27	28	32	31	NEG	NEG	NEG
29	87	F	URTI	5	Oui	0.557 (D6)	Non	25	30	NEG	NEG	NEG	ND	ND
17	44	F	URTI	6	Oui	0.519 (D6)	Non	30	ND	29	26	32	26	31
27	63	F	URTI	8	Oui	0.319 (D4)	Non	34	NEG	30	NEG	NEG	NEG	NEG
22	24	F	URTI	10	Oui	0.723 (D6)	Non	28	NEG	32	34	NEG	NEG	NEG
24	85	F	LRTI	1	Oui	0.619 (D6)	Non	17	21	23	21	26	24	24
23	81	F	LRTI	2	Oui	0.591 (D6)	Non	22	21	30	NEG	32	28	NEG
34	20	M	URTI	2	Oui	0.381 (D6)	Oui	27	31	29	NEG	NEG	NEG	NEG
32	48	M	URTI	2	Oui	0.570 (D6)	Oui	23	29	29	NEG	NEG	NEG	NEG
36	60	M	LRTI	4	Oui	0.319 (D4)	Oui	29	31	31	NEG	NEG	NEG	NEG
33	50	F	LRTI	5	Oui	0.827 (D6)	Oui	30	27	NEG	NEG	NEG	NEG	NEG
35	54	M	LRTI	6	Oui	0.366 (D4)	Oui	24	ND	ND	29	NEG	NEG	NEG
31	53	F	LRTI	7	Oui	1.076 (D6)	Oui	28	31	34	NEG	34	NEG	NEG

This is better, but we are still going to improve it, don't worry

The black line separates the groups, the control group at the top, and the group with chloroquine at the bottom. We simply sorted out the asymptomatic, the URTI with cough and the LRTI with pneumonia.

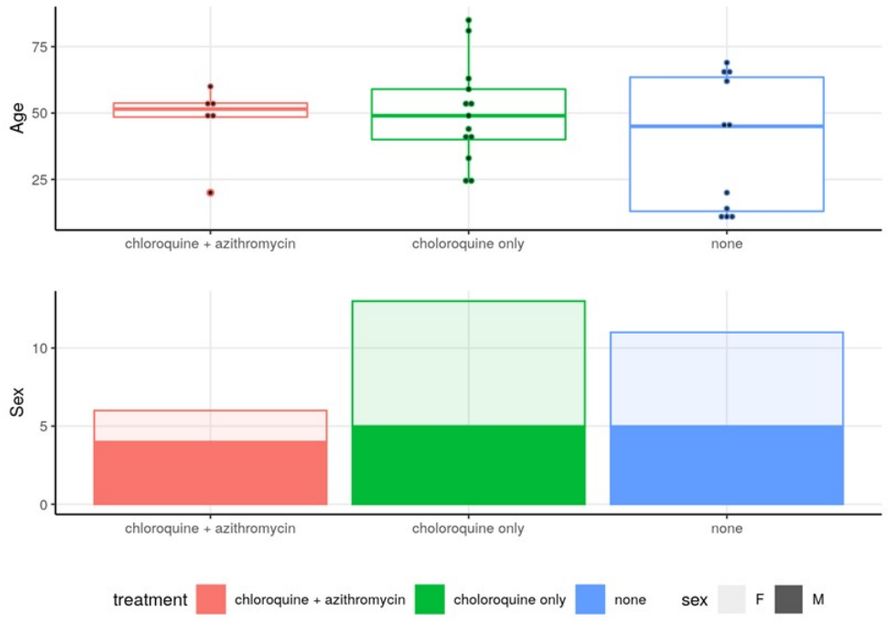
First remark: the groups are not homogeneous :

A/ neither as regarding the age :

Table 1 Characteristics of the study population.

	Age (years)		
	Mean ± SD	t	p-value
Hydroxychloroquine treated patients (N=20)	51.2 ± 18.7	-1.95	0.06
Control patients (N=16)	37.3 ± 24.0		
All patients (36)	45.1 ± 22.0		

37 years old versus 51 years old – with no evidence of which group has an advantage in terms of the rate of viral load decline. (source)



Age and gender distribution in the groups

B/ nor as regarding their clinical status :

Table 1 Characteristics of the study population.

	Clinical status			
	Asymptomatic	URTI	LRTI	p-value
Hydroxychloroquine treated patients (N=20)	2 (10.0)	12 (60.0)	6 (30.0)	0.30
Control patients (N=16)	4 (25.0)	10 (62.5)	2 (12.5)	
All patients (36)	6 (16.7)	22 (61.1)	8 (22.2)	

Let's make sure we do note that there are **25% asymptomatic in the control group.**

Second remark: many data points are missing (the black boxes). Indeed, in the centres that are not in Marseille, and which include the majority of the control group, **the tests are not done on a daily basis**.

And we even observe that **5 patients in the control group are not tested on the 6th day**, which is astounding, since it is the day of the assessment of the **major criterion of judgement** ! The investigators arbitrarily counted these 5 patients as still positive. This is not at all rigorous.

A sixth patient (from the chloroquine group this time) was also not tested in Marseille on days 5 and 6 (light grey). But as he was negative on days 2, 3 and 4, one can reasonably assume that he is still negative. But again, this is not serious: why was he not tested at the IHU, since, not lost sight of, he was obviously still in hospital ?

3-5 The problem of how reliable the tests are

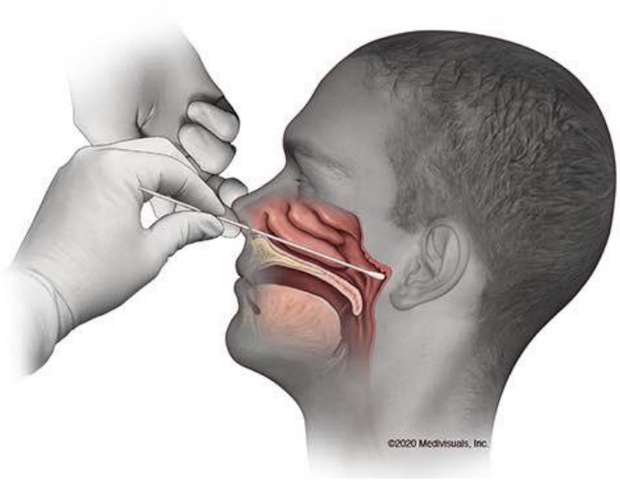
Didier Raoult's choice was to carry out a clinical trial not on the question « Will chloroquine help to treat and protect serious cases in order to save lives » but on the question « Does chloroquine help the organism get rid of the virus at the back of the nose more quickly ». In fact, he removed all severe deteriorations from the study.

So his test basically measures how fast the virus is cleared from the nose (the term « cure » he uses is a bit hasty, there's nothing to say that there isn't some virus left elsewhere in the body – but never mind) – and he does this by testing people who are sick.

These are the famous tests that Raoult is always talking about. But, as usual, Raoult doesn 't say everything, far from it.

To put it simply, he has 2 main steps: 1/ sampling 2/ PCR analysis

The sampling is not easy to do: the swab has to go to the far end of the nasopharynx to soak up the secretions, and it is uncomfortable. This is a major cause for failure – even when it is done by professionals – because if it's not done properly, you won't be able to take enough virus, and the result will be falsely negative.



PCR (Polymerase Chain Reaction) is the laboratory technique for nucleic acid amplification. Let's schematize. We take the virus sample from the swab and put it into the PCR machine. A primer, which is a short piece of the genetic code of the RNA of the virus in mirror (we can use different ones) is introduced; the primer will stick on the corresponding code of the virus; we will then multiply this duplicate, and recover twice the amount of genetic code Q (PCR 1). We run the machine again: at PCR 2, we have 4 times the quantity Q. At PCR 3, we have 8 times the quantity Q, etc. There is an exponential growth. There is a moment when there is such a mass of virus that we will be able to detect it (by fluorescence), because it exceeds the mass M, which is the detection capacity (remember, this is a rough diagram for the general public here).

The measure of viral load shown in the charts is not « a weight of virus » but the number of times the PCR must be run to reach the « M » detection limit. This number of cycles is called the final CT = « cycle threshold » and therefore represents the point at which the signal is significantly greater than the background noise, i.e. the minimum number of cycles needed for the amplified virus RNA to be detectable. (For PCR enthusiasts, you may wish to refer to this dedicated post on Wikipedia).

Then you still have to define a threshold to stop the PCR, in order to declare that at that moment, without RNA being detected, it is assumed that no RNA was present in the sample. Typically, this threshold is defined using positive and negative control samples, and is dependent on the PCR primers that are selected. This work is rarely reported in the methods for a publication, and the scientific community most often trusts this development as long as the results appear to be consistent. However, when the results seem to be inconsistent, for example when the virus disappears and then reappears as if by magic, there is reason to doubt the quality of this above development and the selection of the detection threshold. Whatever

the threshold chosen, there will always be false negatives and false positives: setting it too low will lead to a sample being too easily considered negative, and setting it too high will lead to a sample being too easily considered positive.

In the analysis of Raoult's trial, this threshold is only specified once, in very small print below the chart with the list of patients (see above) it is 35 :

(CT value ≥35).

Let us underline, for the follow-up, that this important information is not clearly included in the dedicated part of the article which talks about PCR (in one line…):

PCR assay

SARS-CoV-2 RNA was assessed by real-time reverse transcription-PCR [17].

nor there

Plates were observed daily for evidence of cytopathogenic effect. Presumptive detection of virus in supernatant was done using SU5000 SEM (Hitachi) then confirmed by specific RT-PCR.

Hence, Raoult's team decided that if, after running the PCR **35 times**, there was still no RNA detection, then the patient no longer had any virus in the back of his nose. But **this threshold is totally arbitrary**: they could have picked 32 or 38.

However, this method also has a **problem of reliability**, which adds to the problem of sampling. And it is not anecdotal – although there is no information on the reliability of the PCR used in Marseille. But we can cite this edifying scientific article (source ; pdf) from March 4th (which we have translated in this post) :

« In this study, we have developed and compared the performance of three new real-time RT-PCR assays targeting the SARS-CoV-2 RNA polymerase (RoRp)/helicase (Hel) genes […] with the RoRp-P2 assay which is used in more than 30 European laboratories. Of the three new tests, the COVID-19-RdRp/Hel assay had the lowest in vitro detection limit […]. Of the 273 specimens from 15 patients with laboratory-confirmed COVID-19 in Hong Kong, 77 (28.2%) were positive for both the COVID-19 RoRp/Hel assay and the RoRp-P2 assay. The COVID-19-OrdRp/Hel assay yielded an additional 42 negative RoRd-P2 specimens [119/273 (43.6%) versus 77/273 (28.2%), P<0.001] ».

To sum up, when 273 samples that you are sure contain the virus are tested using the widely used « RoRp-P2 » PCR, the result is positive in only 28% of the cases – that's 72% false negatives! Of course laboratories use different methods to greatly reduce inaccuracy…

Let us also mention this article from the American CDC (source ; pdf) :

- Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.

> *« **Negative results are not sufficient to rule out Covid-19 infection** and should not be used as the sole basis for treatment or other decisions regarding patient care. A false negative result may occur if a specimen is improperly collected, transported, or handled. **False negative results may also occur** if amplification inhibitors are present in the specimen or if insufficient organisms are present in the specimen. …] Positive and negative predictive values are highly dependent on prevalence. **False negative test results are more likely when the prevalence of the disease is high**. « [CDC, March 30, 2020]*

But let's not be too harsh about the reliability of PCR testing, it's really a fantastic tool. Just think: we discovered the virus less than 3 days ago; we sequenced it in 2 weeks, and were able to have PCR tests less than a month later, and in France thousands of them can be carried out per day. Of course, this was not enough at the beginning of the epidemic, one more mistake of our country. But if we were in 1960, we would still be wondering what this weird virus was made of…

Lastly, we refer the reader to this post quoting Professor Vincent Thibault, head of the virology laboratory at Rennes University Hospital, who explains on France Bleu radio station that **coronavirus screening tests are only 70% reliable**. « In the case of this young girl [16 years old, who died from Covid-19] it seems that two initial samples were negative. She was sent home and her condition deteriorated rapidly with a result that finally turned out positive. This sad case illustrates the problem we face today. » And, last but not least, he adds:

> *« Today we do nasal swabs, but we know that the virus is not in the nose at everystage of the disease (…) **A test can therefore be negative although the patient is symptomatic and indeed contaminated. This is because the virus is located much deeper, in the lungs** for example."*

This sentence totally invalidates the very core of the Gautret / Raoult protocol – whose acceptance by the authorities is all the more surprising.

Indeed, **the nasal viral load of the most severely contaminated patients could very well decrease, because the virus would in fact be migrating into the lungs** (as such was perhaps the case for the patient that was judged and presented as « negative »). It is therefore baffling that the protocol did not include a clinical status of the patients on day 6 – so that it was possible to make sure they were cured, and not on their way to the resuscitation unit… Another serious scientific blunder.

Does anyone know if Didier Raoult ever mentioned this « minor » problem of non-reliability of the tests?

3-6 Analysis of the hydroxychloroquine results

This problem appears very clearly in the Raoult paper:

Patient	Age	Sexe	Statut clinique	Durée entre 1er symptomes et inclusion	Hydroxy-chloroquine	Hydroxy-chloroquine concentration µg/ml (jour de dosage)	Azithromicine	D0	D1	D2	D3	D4	D5	D6
1	10	M	e	--	Non	--	Non	31	NEG	NEG	NEG	NEG	NEG	NEG
4	10	M	e	--	Non	--	Non	24	NEG	33	33	NEG	NEG	32
2	12	F	e	--	Non	--	Non	26	ND	33	34	NEG	34	NEG
3	14	F	e	--	Non	--	Non	26	31	23	22	27	NEG	26
13	45	F	URTI	Inconnu	Non	--	Non	POS	ND	POS	ND	POS	ND	POS
7	46	M	URTI	Inconnu	Non	--	Non	28	ND	ND	ND	26	ND	30
10	66	F	URTI	0	Non	--	Non	POS	ND	POS	ND	ND	ND	POS
14	16	M	URTI	2	Non	--	Non	POS	ND	POS	ND	ND	POS	ND
6	65	F	URTI	2	Non	--	Non	POS	ND	POS	ND	POS	ND	POS
11	75	F	URTI	3	Non	--	Non	POS	ND	POS	ND	POS	ND	ND
5	20	M	URTI	4	Non	--	Non	24	24	24	27	NEG	31	29
12	23	F	URTI	5	Non	--	Non	ND	ND	POS	ND	POS	ND	ND
15	42	F	URTI	5	Non	--	Non	ND	ND	ND	POS	ND	POS	ND
16	23	F	URTI	6	Non	--	Non	POS	ND	ND	ND	ND	POS	ND
8	69	M	LRTI	2	Non	--	Non	POS	ND	POS	ND	POS	POS	POS
9	62	F	LRTI	10	Non	--	Non	POS	ND	POS	ND	POS	ND	POS
18	54	M	Asymptomatiqu	--	Oui	0.462 (D6)	Non	29	NEG	NEG	NEG	NEG	NEG	NEG
20	59	F	Asymptomatiqu	--	Oui	0.288 (D4)	Non	30	NEG	NEG	NEG	NEG	NEG	NEG
28	42	F	URTI	1	Oui	0.453 (D6)	Non	19	16	17	17	19	20	31
21	49	F	URTI	1	Oui	0.621 (D6)	Non	34	27	19	16	34	24	22
30	33	M	URTI	2	Oui	0.194 (D2)	Non	15	23	26	26	ND	32	32
19	25	M	URTI	3	Oui	0.419 (D6)	Non	23	25	28	25	NEG	NEG	NEG
25	40	M	URTI	3	Oui	0.418 (D6)	Non	22	ND	28	21	15	20	17
26	53	M	URTI	5	Oui	0.515 (D6)	Non	27	28	32	31	NEG	NEG	NEG
29	87	F	URTI	5	Oui	0.557 (D6)	Non	25	30	NEG	NEG	NEG	ND	ND
17	44	F	URTI	6	Oui	0.519 (D6)	Non	30	ND	29	26	32	26	31
27	63	F	URTI	8	Oui	0.319 (D4)	Non	34	NEG	30	NEG	NEG	NEG	NEG
22	24	F	URTI	10	Oui	0.723 (D6)	Non	28	NEG	32	34	NEG	NEG	NEG
24	85	F	LRTI	1	Oui	0.619 (D6)	Non	17	21	23	21	26	24	24
23	81	F	LRTI	2	Oui	0.591 (D6)	Non	22	21	30	NEG	32	28	NEG
34	20	M	URTI	2	Oui	0.381 (D6)	Oui	27	31	29	NEG	NEG	NEG	NEG
32	48	M	URTI	2	Oui	0.570 (D6)	Oui	23	29	29	NEG	NEG	NEG	NEG
36	60	M	LRTI	4	Oui	0.319 (D4)	Oui	29	31	31	NEG	NEG	NEG	NEG
33	50	F	LRTI	5	Oui	0.827 (D6)	Oui	30	27	NEG	NEG	NEG	NEG	NEG
35	54	M	LRTI	6	Oui	0.366 (D4)	Oui	24	ND	ND	29	NEG	NEG	NEG
31	53	F	LRTI	7	Oui	1.076 (D6)	Oui	28	31	34	NEG	34	NEG	NEG

Look at the lines framed in red: **some patients are positive one day, negative the next day, and positive again the day after !**

Let's consider the first one, patient no 4 on days 0, 1 and 2: « 24 / NEG / 33 ». Since NEG means 35, this means that this patient's viral load would have been divided by about 2,000 (211) and then, the next day, would have been multiplied by about 4…

The measures framed in blue are very surprising, for instance, for patient 21 (2nd blue-framed line) the recorded values are supposed to be « 16 /34 /24 », which means that the viral load has been divided by about 250,000 (218) within one day and then multiplied by about 1,000 (210) the following day…

In short, the measurements do not appear very reliable. And again, this is blindingly obvious when you study the chart of the results published by the team:

Table 2. Proportion of patients with virological cure (negative nasopharyngeal PCR) by day, in COVID-19 patients treated with hydroxychloroquine and in COVID-19 control patients.

	Day3 post inclusion			Day4 post inclusion			Day5 post inclusion			Day6 post inclusion		
	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value
Hydroxychloroquine treated patients (N=20)	10/20	50.0	0.005	12/20	60.0	0.04	13/20	65.0	0.006	14/20	70.0	0.001
Control patients (N=16)	1/16	6.3		4/16	25.0		3/16	18.8		2/16	12.5	

[a]control patients from centers other than Marseille did not underwent daily sampling, but were sampled every other day in most cases, they were

Note: A reviewer who has repeated the calculations reports that he finds P-values that are twice as high (source). This should be checked.

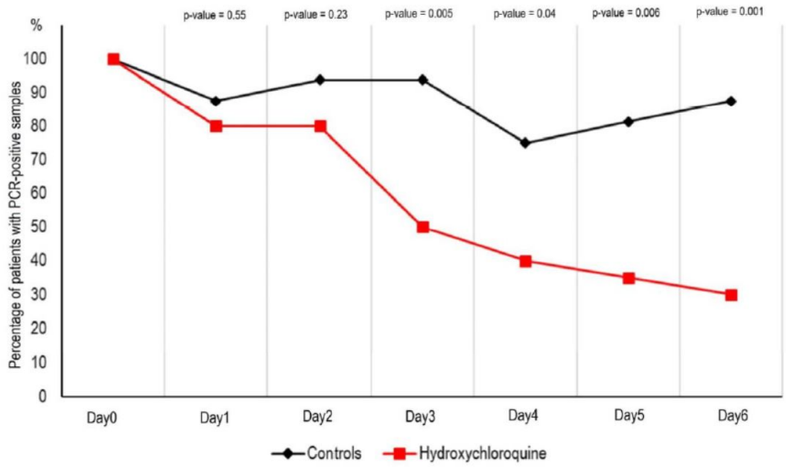
So there's one scientist who wrote without batting an eyelid – and 17 others are supposed to have reviewed before signing – that the control group had :

- 1 negative out of 16 on day 3;
- 4 negatives out of 16 on day 4;

- but 3 negatives out of 16 on day 5;
- and thus only 2 negatives out of 16 on day 6 (the day of the result of this study).

And this is clearly visible on their graph, which was widely circulated when the study was published :

Figure 1. Percentage of patients with PCR-positive nasopharyngeal samples from inclusion to day6 post-inclusion in COVID-19 patients treated with hydroxychloroquine and in COVID-19 control patients.



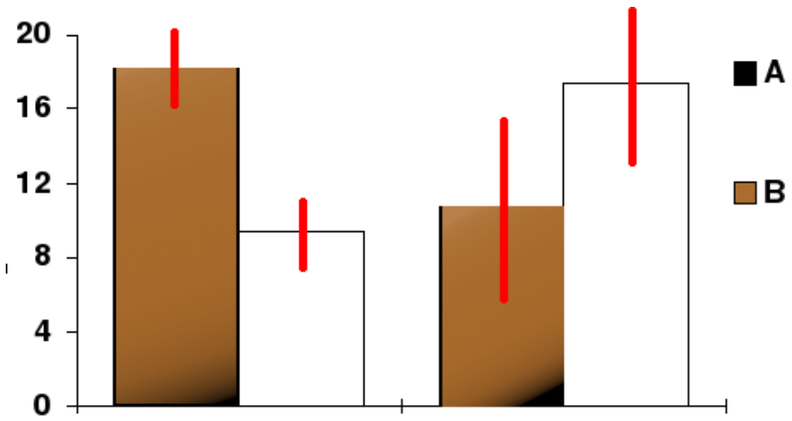
Everyone focused on the difference between the curves, without taking any notice of the fact that **the percentage of positives increased 3 times in the control group**, which is obviously ridiculous!

Here is another problem. As we have seen, many missing measurementsare missing for the control group (the famous « NDs » on the black background).

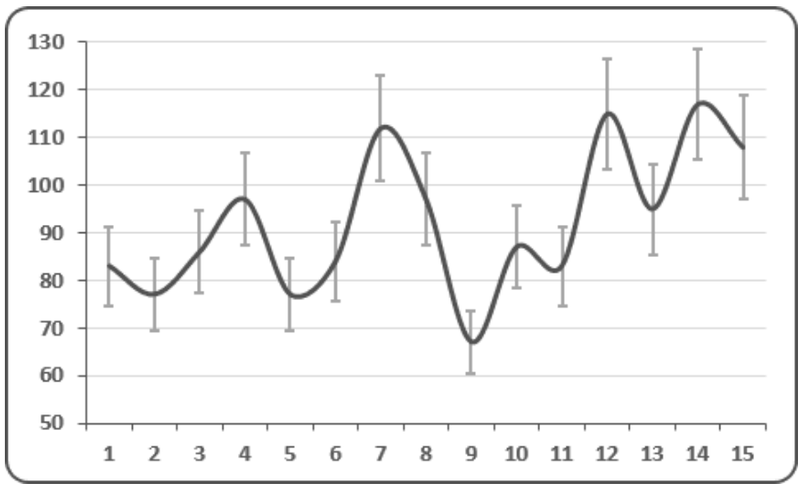
All scientists know that the results must be analysed in light of the statistical uncertainty of the result measurement.

Let's take an example. Let's imagine that we want to determine the average age of the Presidents of the French Republic on the day of their first election. With only one value, for Macron, the average is **39**. With 2 values (Holland, 57) the average is **48**. With Sarkozy (52) the average is **49**. When including the 8 presidents of the Fifth Republic, we reach an average of 56. With all the others, we reach the real value of **60**. It is therefore obvious that the robustness of the 39- year average corresponding to the Macron measure alone is much lower than that of the 56-year average corresponding to 8 values. The more measurements there are, the closer we get to the correct value.

To reflect this, scientists use **error bars** which are graphical representations of the variability of data and are used on graphs to indicate the error, or uncertainty in a reported measurement. They give a general idea of the accuracy of the measurement, or conversely, how far away from the reported value the true value is. In most cases, error bars represent a standard deviation of uncertainty, a standard error, or a certain confidence interval (e.g. a 95% confidence interval) (source: Wikipedia). See the following 2 examples for a better understanding:



Here the measurements displayed as histograms are accompanied by a confidence interval (in red) indicating the amplitude where the actual value lies with a probability of, let's say, 95%. The height depends on the size of the sample on which the measurement is made (it would be very large for Macron's 39 years, but much smaller for the 56 years of the 8 measurements).

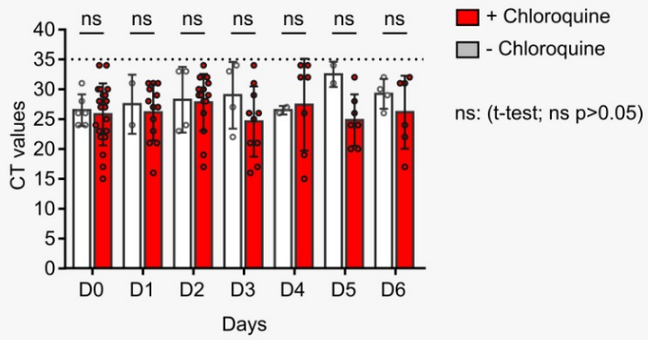


Similarly, the above graph shows the theoretical variability of the curve measurements.

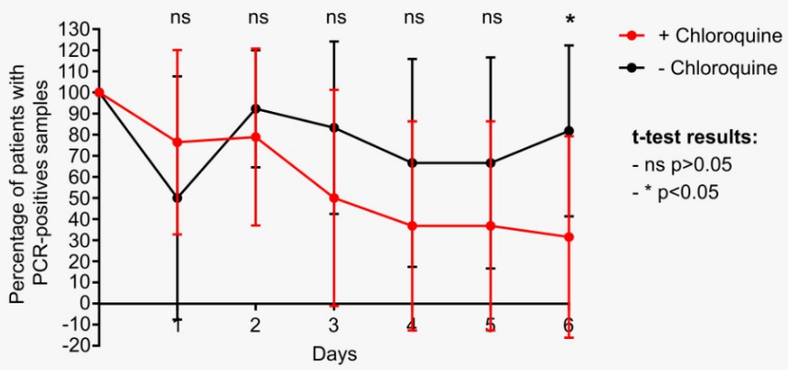
To make a long story short, a PubPeer contributor (source) presented the uncertainty that should have been included in Raoult's paper as follows:

Data from:
Gautret et al. (2020) Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents In Press 17 March 2020 – DOI : 10.1016/j.ijantimicag.2020.105949

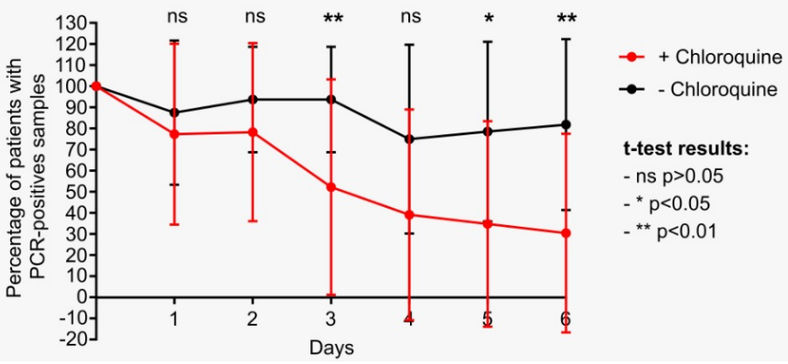
Graph of PCR results presented in table 1:



Graph presenting % of positive patients (ND values not analysed)



Graph presenting % of positive patients (ND values considered as positive if PCR was positive the following days)



It appears clearly that the sample is so small that the uncertainty bars are overlapping across the two curves, which means that they have absolutely no statistical robustness, and that the differences between the curves may be as much the result of chance as of treatment.

Problem: The sample is far too small to enable any conclusion to be drawn.

And that's not all !

3-7 Results for Hydroxychloroquine and Azithromycin

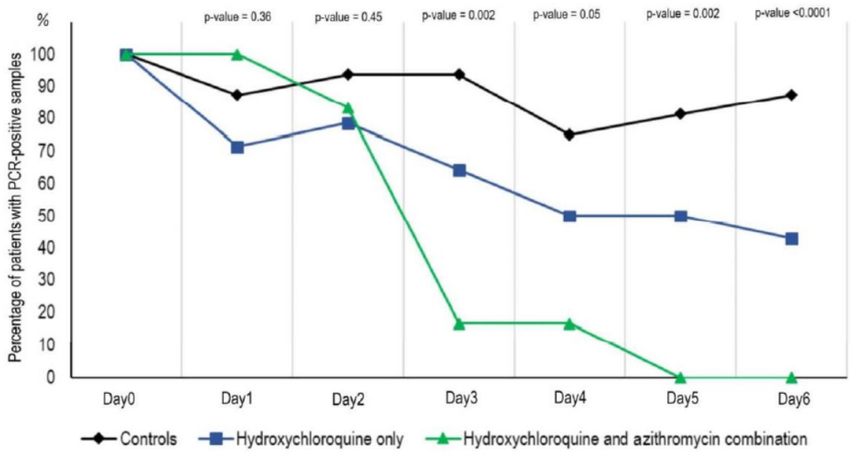
We'll come back to this in the next post, but it should be noted that Raoult also tested a hydroxy-chloroquine + azithromycin (antibiotic) combination, with the following results:

When comparing the effect of hydroxychloroquine treatment as a single drug and the effect of hydroxychloroquine and azithromyc in combination, the proportion of patients that had negative PCR results in nasopharyngeal samples was significantly different between the two groups at days 3-4-5 and 6 post-inclusion (Table 3). At day6 post-inclusion, 100% of patients treated with hydroxychloroquine and azithromycin combination were virologicaly cured comparing with 57.1% in patients treated with hydroxychloroquine only, and 12.5% in the control group ($p<0.001$). These results are summarized in Figures 1 and 2. Drug effect was significantly higher in patients with symptoms of URTI and LRTI, as compared to asymptomatic patients with $p<0.05$ (data not show).

Table 3. Proportion of patients with virological cure (negative nasopharyngeal PCR) by day, in COVID-19 patients treated with hydroxychloroquine only, in COVID-19 patients treated with hydroxychloroquine and azithomycin combination, and in COVID-19 control patients.

	Day3 post inclusion			Day4 post inclusion			Day5 post inclusion			Day6 post inclusion		
	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p-value
Control patients	1/16	6.3		4/16	25.0		3/16	18.8		2/16	12.5	
Hydroxychloroquine treatment only	5/14	35.7	0.002	7/14	50.0	0.05	7/14	50.0	0.002	8/14	57.1	<0.001
Hydroxychloroquine and azithromycin combined treatment	5/6	83.3		5/6	83.3		6/6	100		6/6	100	

Figure 2. Percentage of patients with PCR-positive nasopharyngeal samples from inclusion to day6 post-inclusion in COVID-19 patients treated with hydroxychloroquine only, in COVID-19 patients treated with hydroxychloroquine and azithomycin combination, and in COVID-19 control patients.



By day 5, all patients treated with Raoult cocktail are cured!

We have to admit that, presented this way, the effectiveness of the treatment seems very convincing indeed.

And there is more to it:

We show here that hydroxychloroquine is efficient in clearing viral nasopharyngeal carriage of SARS-CoV-2 in COVID-19 patients in only three to six days, in most patients. A significant difference was observed between hydroxychloroquine-treated patients and controls starting even on day3 post-inclusion. These results are of great importance because a recent paper has shown that the mean duration of viral shedding in patients suffering from COVID-19 in China was 20 days (even 37 days for the longest duration) [19]

This has to be read carefully:

1/ « **hydroxychloroquine is efficient in clearing viral nasopharyngeal carriage (…) in only three to six days, in most patients.**«

It is quite unbelievable to draw such definitive conclusions on such a small sample in a trial full of biases;

2/ This difference with « **control group starts even as early as day 3 post-inclusion**«

This is, indeed, quite remarkable, considering the fact that this control group is very poorly tested on a daily basis:

Patient	Age	Sexe	D0	D1	D2	D3	D4	D5	D6
1	10	M	31	NEG	NEG	NEG	NEG	NEG	NEG
4	10	M	24	NEG	33	33	NEG	NEG	32
2	12	F	26	ND	33	34	NEG	34	NEG
3	14	F	26	31	23	22	27	NEG	26
13	45	F	POS	ND	POS	ND	POS	ND	POS
7	46	M	28	ND	ND	ND	26	ND	30
10	66	F	POS	ND	POS	ND	ND	ND	POS
14	16	M	POS	ND	POS	ND	ND	POS	ND
6	65	F	POS	ND	POS	ND	POS	ND	POS
11	75	F	POS	ND	POS	ND	POS	ND	ND
5	20	M	24	24	24	27	NEG	31	29
12	23	F	ND	ND	POS	ND	POS	ND	ND
15	42	F	ND	ND	ND	POS	ND	POS	ND
16	23	F	POS	ND	ND	ND	ND	POS	ND
8	69	M	POS	ND	POS	ND	POS	POS	POS
9	62	F	POS	ND	POS	ND	POS	ND	POS

3/ These results are « of great importance because a recent paper has shown **that the mean duration of viral shedding (…) in China was 20 days** (even 37 days for the longest duration) ».

First of all, it is odd that a small sample treated in Marseilles be compared in a clinical trial with a population hospitalised in China 2 months earlier – it is not even certain, for instance, that the strain of virus is the same…

This is yet another proof of the lack of seriousness attached to such an important study. One reviewer (source) pointed out that the previous graph is not identical to the one presented by Raoult on March 16 th, during his presentation (in his video, orhere, archive, source here, archive):

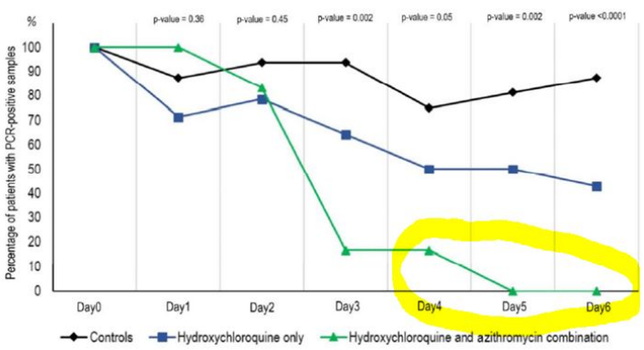
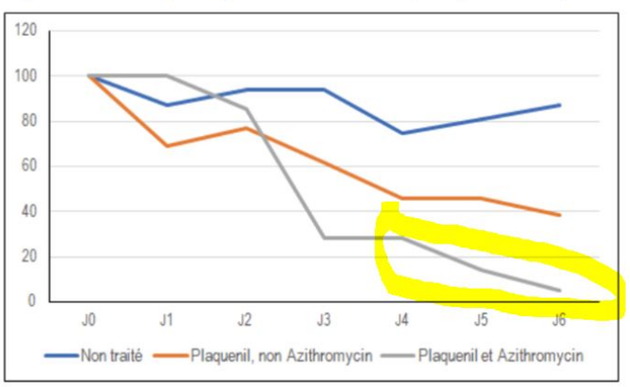


Figure 2: Pourcentage des positifs entre Non traité, PLQ seul et PLQ + AZT



However, it is certain that if the treatment was able to suppress the viral carriage « in just 3 to 6 days », when the average duration « in China was 20 days », it would be spactacular.

If it were true…

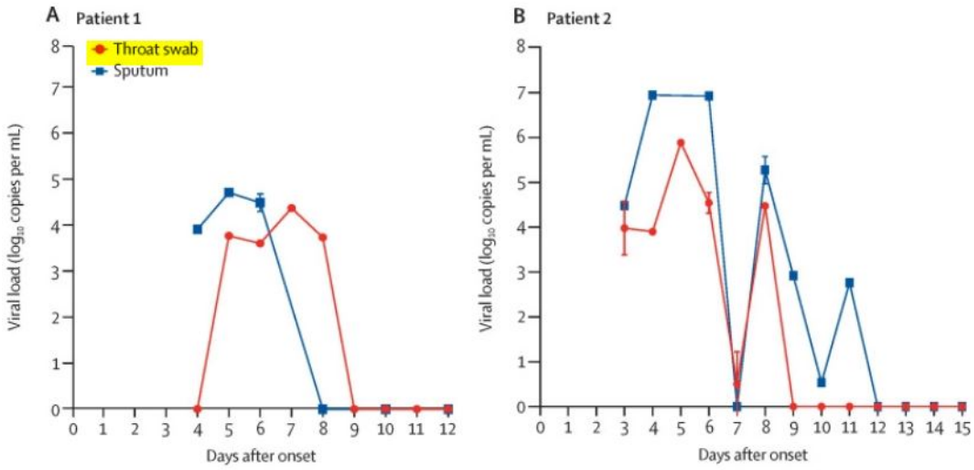
3-8 Viral carriage

This section, although it may seem a bit technical, is crucial to the understanding of a very serious bias in the study.

3-8-1 Overview

The purpose of this trial is therefore to compare the speed with which the virus can be evacuated from the back of the nose – also called viral carriage.

This study, published by the Lancet in February, traced the evolution of the viral load in two Chinese patients, in their throat (in red) and sputum (in blue):



There clearly is a measurement abnormality on day 7, on the right – the red line of the patient on the left (patient 1) is more easily readable.

We see that the viral load starts increasing in the nose between the 2nd and the 4th day, reaches a peak between day 5 and day 7, and for those 2 patients disappears on day 9 – which doesn't necessarily mean that those patients were heaaled at that moment.

Thus, as we want to know about the speed of viral shedding among patients, we must take into acount the moment at which we start monitoring the patient: the shedding duration will be much shorter if you start monitoring the patient on the 7th day after the onset of the symptoms than if you start on the 2nd day…

This is why the analysis specifies that on average, the patients join the trial 4 days after the onset of symptoms..

Table 1 Characteristics of the study population.

	Time between onset of symptoms and inclusion (days)		
	Mean ± SD	t	p-value
Hydroxychloroquine treated patients (N=20)	4.1 ± 2.6	-0.15	0.88
Control patients (N=16)	3.9 ± 2.8		
All patients (36)	4.0 ± 2.6		

This means that the patients are monitored, at the end of the trial, 10 days after the onset of symptoms

This would be half of the duration of the viral shedding in China; the amount of negative tests would then be a great result for the treatment.

« WOULD BE »…

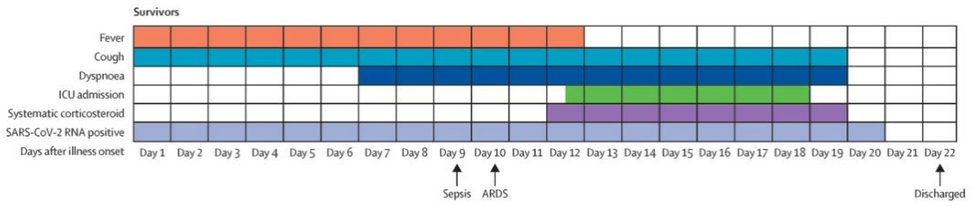
3-8-2 « The average duration of viral carriage […] in China was 20 days

This information about the average duration of 20 days is a key point in the argumentation of Raoult article. The reference is to be found in this study (there):

[19] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 11. pii: S0140-6736(20)30566-3. doi: 10.1016/S0140-6736(20)30566-3.

This article was published on March 11 th (and is therefore quoted in Raoult article on March 16 th) and indeed says :

For survivors, the median duration of viral shedding was 20·0 days (IQR 17·0–24·0) from illness onset, but the virus was continuously detectable until death in non-survivors (table 2; figure 1). The shortest observed duration of viral shedding among survivors was 8 days, whereas the longest was 37 days. Among 29 patients who received lopinavir/ritonavir and were discharged, the median time from illness onset to initiation of antiviral treatment was 14·0 days (IQR 10·0–



Let's first observe that if the duration of viral shedding for this group composed of hundreds of hospitalized Chinese people is indeed 20 days (between 17 and 24, to be precise), ranging from 8 to 37 days, the article mentions the median duration (it's longer for half of the patients, shorter for the other half) and not the **average duration**, those are different figures.

Problem: Raoult team merges average durations and median durations.

However, the study calls for caution regarding the robustness of this data:

> *« the estimated duration of viral shedding is limited by the frequency of respiratory specimen collection, lack of quantitative viral RNA detection, and relatively low positive rate of SARS-CoV-2 RNA detection in throat-swabs »*
>
> *« the estimated duration of viral shedding is limited by the frequency of respiratory specimen collection, lack of quantitative viral RNA detection, and relatively low positive rate of SARS-CoV-2 RNA detection in throat-swabs »*

But actually, that's not the point. Because in order to average viral shedding, youneed to have the measurements history of the patients who are no longer carrying the virus. And therefore, we need to define when these patients are no longer carrying the virus. We therefore need to look into the methodology of the study – which actually refers to another study (available here):

Laboratory procedures

Methods for laboratory confirmation of SARS-CoV-2 infection have been described elsewhere.[5]

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

nCoV. The presence of 2019-nCoV in respiratory specimens was detected by next-generation sequencing or real-time RT-PCR methods. The primers and probe target to envelope gene of CoV were used and the sequences were as follows: forward primer 5′-ACTTCTTTTTCTTGCTTTCGTGGT-3′; reverse primer 5′-GCAGCAGTACGCACACAATC-3′; and the probe 5′CY5-CTAGTTACACTAGCCATCCTTACTGC-3′BHQ1. Conditions for the amplifications were 50°C for 15 min, 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 30 s.

The Chinese scientists aim at the envelope gene of the virus, during 45 cycles.

So they have a very thorough search for traces of the virus. Which is not of the same nature or level as the one carried out in Marseilles…

Therefore, one cannot compare average durations and median durations on such different bases!

3-8-3 The Marseilles case

As we've seen, the article refers to another article on the methodology of the PCR technique that has been used (it's here)

PCR assay

SARS-CoV-2 RNA was assessed by real-time reverse transcription-PCR [17].

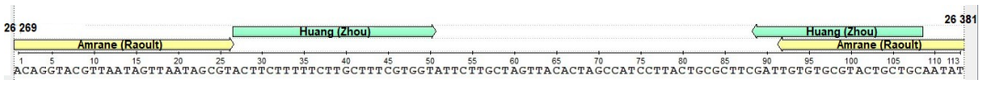
[17] Amrane S, Tissot-Dupont H, Doudier B, Eldin C, Hocquart M, Mailhe M et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious diseases referral hospital in Marseille, France, - January 31st to March 1st, 2020: A respiratory virus snapshot. Travel Med Infect Dis. 2020 [Epub ahead of print].

The article indicates the ARN primer of the virus,

2.3. Laboratory tests

Viral RNA was extracted from 200 µL of naso- and oro-pharyngeal swab fluid and/or sputum, using the EZ1 Virus Mini Kit v2.0 (Qiagen®, Courtaboeuf, France). For the detection of SARS-CoV-2 RNA we used two different RT-PCR systems with a hydrolysis probe and the LightCycler Multiplex RNA Virus Master kit (Roche Diagnostics®, Mannheim, Germany). The first system targets the envelope protein (E)-encoding gene and was previously described [7] and uses a synthetic RNA positive control (supplied by the Charité virology institute - Universitätsmedizin Berlin, Berlin, Germany [8]). The second system was designed in-house, targets the spike protein-encoding gene (forward primer: 5′-AAACTTGTGCCCTTTTGGTG-3′; reverse primer: 5′-TGCTGATTCTCTTCCTGTTCC-3′; probe: 5′-CGCCACCAGATTTGCATCTG-3′), and uses a synthetic RNA positive control ordered from Eurogentec® (Seraing, Belgium). In some cases, a real-time RT-PCR was carried out with the QuantiNova SYBR Green RT-PCR kit (Qiagen®) that targeted either the E gene with the same primers as above or previously described primers targeting the RNA-dependent RNA polymerase (RdRp)-encoding gene [7] with a synthetic RNA positive control (Eurogentec). A phage RNA internal control [9] was added to each clinical sample before extraction to ensure RNA extraction and PCR amplification were accurate. All experiments were performed on a LightCycler 480 instrument (Roche Diagnostics) by trained qualified technicians available who were available around the clock, either during routine working days and hours, or on an on-call basis. Concurrently, a multiplex molecular assay that detects respiratory pathogens was performed at the point-of-care laboratory [10] with the Biofire FilmArray Respiratory panel 2 test (Biomérieux, Marcy-l'Etoile, France) or the FTD Respiratory pathogens 21 kit (Fast Track Diagnosis, Luxembourg).

We've also checked: The team from Marseilles has simply shifted the Chinese primer :



The PR will replicate the part of the genetic code of the virus displayed here, between the primers (arrows)

Unfortunately, the article doesn't mention the number of cycles, refering to another article (here) :

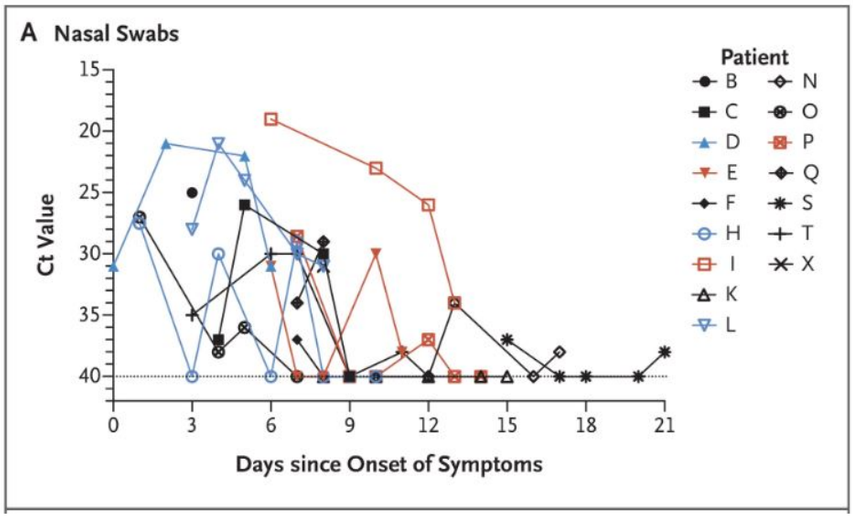
We finally discover that, it seems, the practice should be to do 45 cycles:

> Real-time reverse-transcription PCR
>
> A 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribont triphosphates (dNTP) and 3.2 mM magnesium sulphate), 1 µL of reverse transcriptase/Taq mixture from the kit, 0.4 µL of a 50 mM magnesium sulphate solution (Invitrogen), and 1 µg of nonacetylated bovine serum albumin (Roche). Primer and probe sequences, as well as optimised concentrations are shown in Table 1. All oligonucleotides were synthesised and provided by Tib-Molbiol (Berlin, Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15 s, 58 °C for 30 s. Participating laboratories used either Roche Light Cycler 480II or Applied Biosystems ViiA7 instruments (Applied Biosystems, Hong Kong, China).

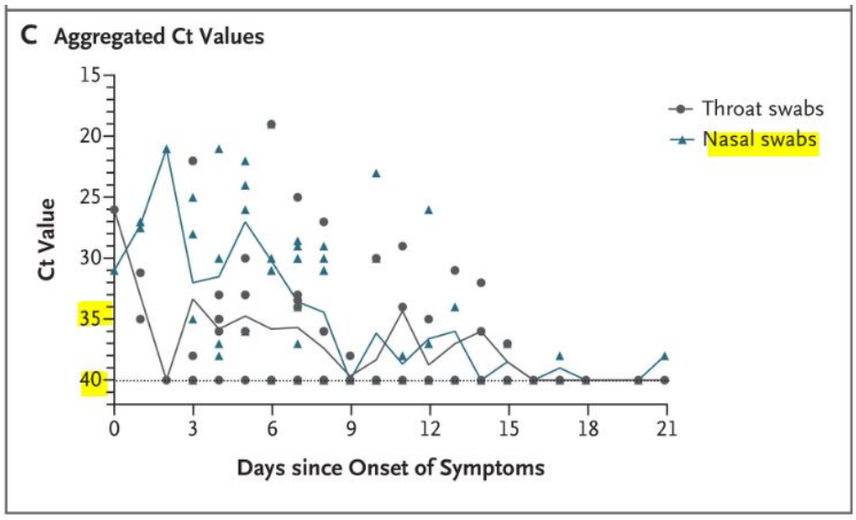
We'll see that this issue is not anecdotal

3-8-4 Another example to make it clear

This excellent article *SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients* (source) dated February 19th gives us interesting informations, from the measures of viral loads of about 15 Chinese patients.



Here is the viral carriage (the higher the number, the less virus there is, the reverse scale is therefore normal). The patients in intensive care are indicated in red, in blue are those whose state is moderate.



The curve of the average viral load according to the cycle threshold (Ct) is shown in blue. This means that a **viral load is indeed observed up to 18-21 days, but this is because the detection threshold is 40 Ct.**

Thus, with a value of 35 Ct (which is not fully comparable to that of Raoult, but should be quite close), **anaverage duration of 8-9 days would probably have been necessary for the virus to disappear. That is to say a little less than in the Marseilles sample treated with chloroquine…**

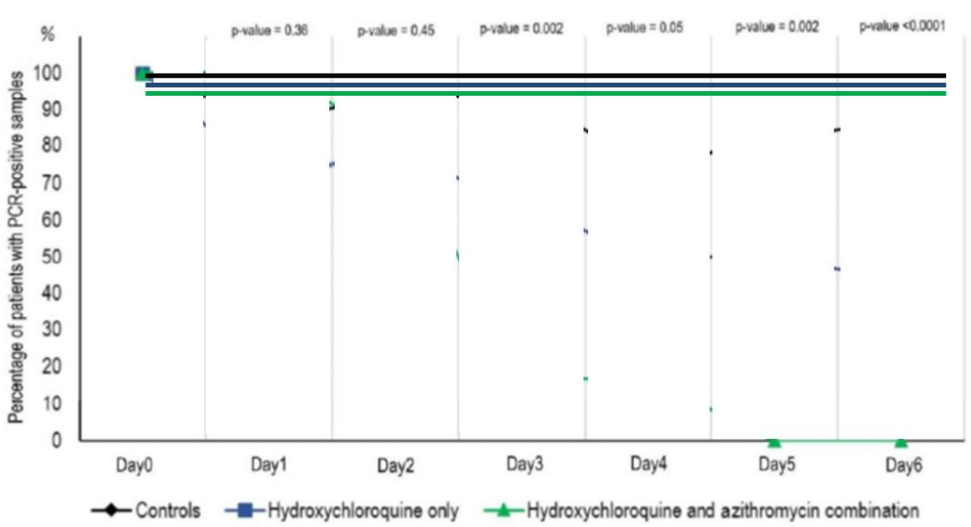
3-8-5 Conclusion

In view of the methodological weakness of the article, it would help if the investigators could:

- specify the primer that has been used;
- confirm the Ct value of 35;
- indicate whether this Ct value is customary in Marseilles – and if not explain why;
- and explain how they can compare their results with those of the Chinese study with a Ct value of 45.

Indeed, it is more than likely that a Ct of 45 would have led to 100% of the patients treated with chloroquine being, like the control group, positive on day 6, invalidating the result.

A Ct value of 45 in Marseilles would probably have led to this:



But we have to admit that this would have made it more difficult to sell to the media.

Let's hope the IHU will give some explanation about this (was the Ct used in the trial a usual practice; why not use a Ct value of 45 as in China since the results were compared with those of the Chinese study) in order to leviate the doubt.

Last remark: it was noted that patients in the control group that were not treated in Marseilles had no Ct value but only a POSitive or NEGative status. It can therefore be assumed that the samples were not analysed in Marseilles but in the laboratories of the various hospitals where they were. Therefore, there is no way of knowing whether they used the same primer and a Ct value of 35 for their PCR.

And ther is also nothing that indicates that all of them detect the same level of virus. If they were finer in their analysis, they would detect the virus forsignificantly longer, which would totally distort the comparison with thechloroquine group treated (and would then account for the discrepancy) – and would therefore make the clinical trial totally useless.

3-9 Let's put some seriousness in this trial…

We are therefore dealing with a trial comparing the speed of recovery of 2 groups, this speed depending on the seniority of the disease.

The article indicates that the the control group, comprising 16 people with a mean time of 3.9 days, is comparable to the treated group:

Table 1 Characteristics of the study population.

	Time between onset of symptoms and inclusion (days)		
	Mean ± SD	t	p-value
Hydroxychloroquine treated patients (N=20)	4.1 ± 2.6	-0.15	0.88
Control patients (N=16)	3.9 ± 2.8		
All patients (36)	4.0 ± 2.6		

But **this is a lie.**

Patient	Age	Sexe	Statut clinique	Durée entre 1er symptomes et inclusion
1	10	M	Asymptomatique	--
4	10	M	Asymptomatique	--
2	12	F	Asymptomatique	--
3	14	F	Asymptomatique	--
13	45	F	URTI	Inconnu
7	46	M	URTI	Inconnu
10	66	F	URTI	0
14	16	M	URTI	2
6	65	F	URTI	2
11	75	F	URTI	3
5	20	M	URTI	4
12	23	F	URTI	5
15	42	F	URTI	5
16	23	F	URTI	6
8	69	M	LRTI	2
9	62	F	LRTI	10

Patients 1, 4, 2 and 3 are asymptomatic; no date of first symptoms is available for patients 13 and 7! Same thing for the 2 in the group treated with chloroquine.

3.9 is a mean value for the 10 other patients in the control group. Not for the 16 patients obviously.

Indeed, it is not scientifically acceptable to include in this trial (on speed of recovery) people whose time of the disease is not known!

Similarly, it is not scientifically acceptable to include 6 patients who have not been tested on day 6 in the evaluation of the trial on day 6 ! As Didier Raoult likes to say, « It's delirious! »

So here is what this little trial would look like once cleared of these abnormalities:

Patient	Age	Sexe	Statut clinique	Durée entre 1er symptomes et inclusion	Hydroxy-chloroquine	Hydroxy-chloroquine concentration µg/ml (jour de dosage)	Azithromicine	D0	D1	D2	D3	D4	D5	D6	
10	66	F	URTI	0	Non	--	Non	POS	ND	POS	ND	ND	ND	POS	
6	65	F	URTI	2	Non	--	Non	POS	ND	POS	ND	POS	ND	POS	
5	20	M	URTI	4	Non	--	Non	24	24	24	27	NEG	31	29	
8	69	M	LRTI	2	Non	--	Non	POS	ND	POS	ND	POS	POS	POS	
9	62	F	LRTI	10	Non	--	Non	POS	ND	POS	ND	POS	ND	POS	
28	42	F	URTI	1	Oui	0.453 (D6)	Non	19	16	17	17	19	20	31	*Jour de*
21	49	F	URTI	1	Oui	0.621 (D6)	Non	34	27	19	16	34	24	22	*Guérison*
30	33	M	URTI	2	Oui	0.194 (D2)	Non	15	23	26	26	ND	32	32	UR LR
19	25	M	URTI	3	Oui	0.419 (D6)	Non	23	25	28	25	NEG	NEG	NEG	7
25	40	M	URTI	3	Oui	0.418 (D6)	Non	22	ND	28	21	15	20	17	
26	53	M	URTI	5	Oui	0.515 (D6)	Non	27	28	32	31	NEG	NEG	NEG	9
29	87	F	URTI	5	Oui	0.557 (D6)	Non	25	30	NEG	NEG	NEG	ND	ND	7
17	44	F	URTI	6	Oui	0.519 (D6)	Non	30	ND	29	26	32	26	31	
27	63	F	URTI	8	Oui	0.319 (D4)	Non	34	NEG	30	NEG	NEG	NEG	NEG	11
22	24	F	URTI	10	Oui	0.723 (D6)	Non	28	NEG	32	34	NEG	NEG	NEG	14
24	85	F	LRTI	1	Oui	0.619 (D6)	Non	17	21	23	21	26	24	24	
23	81	F	LRTI	2	Oui	0.591 (D6)	Non	22	21	30	NEG	32	28	NEG	8
34	20	M	URTI	2	Oui	0.381 (D6)	**Oui**	27	31	29	NEG	NEG	NEG	NEG	5
32	48	M	URTI	2	Oui	0.570 (D6)	**Oui**	23	29	29	NEG	NEG	NEG	NEG	5
36	60	M	LRTI	4	Oui	0.319 (D4)	**Oui**	29	31	31	NEG	NEG	NEG	NEG	7
33	50	F	LRTI	5	Oui	0.827 (D6)	**Oui**	30	27	NEG	NEG	NEG	NEG	NEG	7
35	54	M	LRTI	6	Oui	0.366 (D4)	**Oui**	24	ND	ND	29	NEG	NEG	NEG	10
31	53	F	LRTI	7	Oui	1.076 (D6)	**Oui**	28	31	34	NEG	34	NEG	NEG	12
														Moyenne :	*8 9*

Unfortunately, there are only 5 people left in the control group (but we will see that Didier Raoult is not really interested in control groups) as compared with 18 in the treated group.

We can, however, calculate the average day of passage in negative, for the negatives: **8th day for URTIs, 9th day for LRTIs**. This is exactly what was shown in the previous Chinese study for a Ct of 35…

Here is, therefore, what remains of this trial – when excluding the numerous above-mentioned biases

		Nombre	Âge	Ancienneté	Négatifs à D6	%
URTI	Contrôle	3	**50**	**2**	0	*0 %*
	HCQ	10	46	4	6	*60 %*
	HCQ+AZM	2	34	2	2	*100 %*
LRTI	Contrôle	2	65	6	0	*0 %*
	HCQ	2	83	**1**	1	*50 %*
	HCQ+AZM	4	54	5	4	*100 %*

When considering the last column alone, the treatment seems to be working very well.

But if you consider all of it:

- for URTIs (bad colds): as the HCQ treatment is 4 days old, and measured on day 6, we measure on day 10 of symptoms; the average is 8 days of recovery, so it is not surprising to have 60% people healed. The subgroup with the antibiotic is 34 years old on average, so these people have probably eliminated the virus more quickly. Remains the control group with no negative; but the « group » consists of 3 people, older than the others, with only 2 days of clearance; with an average of 8 days of clearance, it is not very surprising to have no negative on the 6th day…

- for LRTI (pneumonia): the HCQ+AZM group is once again the youngest of the groups; it has 5 days of seniority, for an average clearance time of 9 days: it is normal to have a good result in this example.The control group only has 2 patients; the one with 2 days of seniority would have just had time to go into negative; the second, starting on day 10, should have gone into negative, starting with a seniority higher than the average, but this was not the case, he may have to go to ICU if his condition worsens.

Let's mention one more thing: the concentration of chloroquine in the body (which is given in the chart) does not seem to have a very strong influence on the speed of shedding.

3-10 But in fact, what was Raoult's objective on March 9?

In this video dated March 9 th, Raoult's objective was as follows:

> *« Our research project on hydroxy-chloroquine has just been accepted, and we're implementing it with **two objectives** :*
>
> - *Point one is **toimprove clinical management**, that is specifically for patients with rather severe symptoms,*
> - *and on the other hand, and that is our second objective, it is to see if we can quickly, because that's what the Chinese said, reduce viral carriage, i.e. when t**he natural viral carriage is apparently around 12 days, Mr. Zhong reported that under chloroquine the viral carriage was reduced to 4 days.***
>
> *And so we do hope to confirm these data because then it will allow, especially for those who carry considerable amounts of virus, to decrease this viral load, and the risk of secondary contamination. « [Didier Raoult, March 9, 2020]*

Thus, on March 9 th, according to Raoult, the average time of portage in China was **12** days, but on the 16th in his paper, he fetched, without a question, a Chinese study that mentions **20** days. And his goal on March 9 th was to bring it down to **4**days, but on the 16th he welcomes a treatment that indicates an average viral carriage of probably **9 to 11** days. Moreover, he does not talk in his article about the improvement of clinical management – but with 1 death and 3 cases of resuscitation out of 26, it is true that there is nothing to be glorious about.

This trial is therefore a **failure** in relation to its initial objectives, which he refrains from saying.

3-11 One last big problem

Back to the patients

> *Clinical classification*
>
> Patients were grouped into three categories: asymptomatic, upper respiratory tract infection (URTI) when presenting with rhinitis, pharyngitis, or isolated low-grade fever and myalgia, and lower respiratory tract infections (LRTI) when presenting with symptoms of pneumonia or bronchitis.

The patients are therefore all hospitalized. We also have that they are classified according to their clinical state :

one patient decided to leave the hospital on day3 post-inclusion and was PCR-negative on days1-2;

But in fact, when is the classification established? On the first day, as it is likely, or on the 6th?

1/ If it's on the 6th day, it means that some patients' status will be different be they on the 6th day or on the 14th.

And why isn't there anywhere a « cured » status? Although there was at least one, the patient, reported as « lost from sight »:

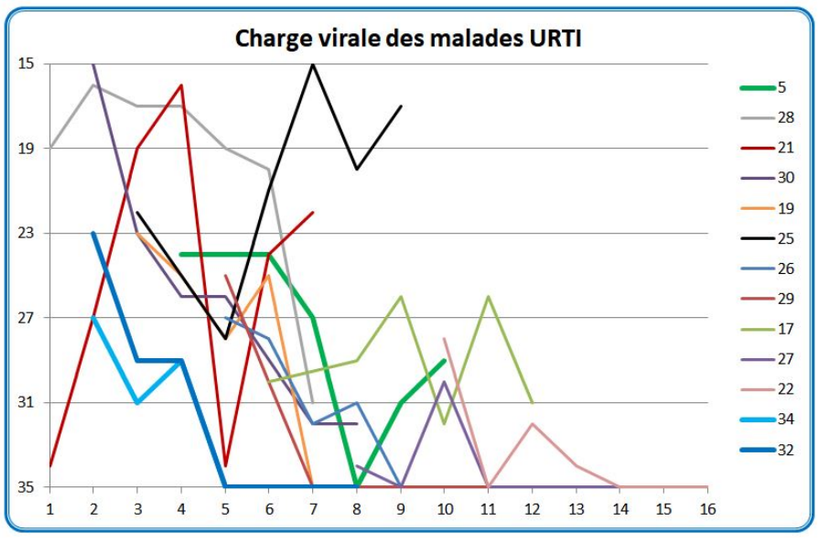
But why on earth is being cured considered as being lost from sight?

Does this mean that, when a patient has a non detected viral load several days in a row, and no more symptoms, the investigators still go on treating them, until the 14th day? Why that?

All patients in Marseille center were proposed oral hydroxychloroquine sulfate 200 mg, three times per day during ten days

2/ If this happens on the first day, then it would make more sense. But then, what happens when the patient's condition worsens? The team would be lucky if, among the 22 UTRI included in a period of time of 0 to 10 days after the onset of symptoms, none worsened into a pneumonia (LTRI)…

We have shown here the evolution of the viral load of the URTI patients (common symptoms of a big cold) measured in CT (from 15 (many viral particles) to 35 (few viral particles)) this depending on the number of days after the onset of symptoms :



The thick green line represents the only URTI patient from the control group; the thick blue lines represent the 2 patients who received the HCQ + antibiotics treatment; all the others received HCQ

It is easy to see that some measures are quite surprising, in zigzag patterns. We do see that although both patients receiving HCQ had a sharp decrease of the viral load (they are however young, 20 and 48 years old); we must also note that they started with a small viral load.

Let's now focus on the LRTI (pneumonia) patients: