LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
LHUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

Facteurs pronostics de la fibrose pulmonaire post-COVID-19

29/09/2022 Corata à Lille

Projet de recherche de Nathalie De Vos au LHUB-ULB

Promoteur Prof. Frédéric Cotton

CONTENT

CONTENT

I. INTRODUCTION

- Pulmonary fibrosis in SARS
  - 2003 CT thorax in N=24 severe SARS-CoV-1 survivors 1 months post-ICU
    - In previous coronavirus outbreaks, studies from SARS-CoV-1 survivors showed that early fibrotic lesions can be seen in the first months of follow up especially in critical and elderly patients
    - Evidence of fibrosis on CT scan: parenchymal band, traction bronchiectasis, irregular interfaces

    Antonio GE, Wong KT, Hui DS, Wu A, Lee N, Yuen EH, Leung CB, Rainer TH, Cameron P, Chung SS, Sung JJ, Ahuja AT. Thin-section CT in patients with severe acute respiratory syndrome following hospital discharge: preliminary experience. Radiology. 2003 Sep;228(3):810-5. doi: 10.1148/radiol.2283030726. Epub 2003 Jun 12. PMID: 12805557
  - 2020 pandemic wave 1: start of this LHUB-ULB study predicting pulmonary fibrosis as a COVID-19 sequela
    - 86% (N=19/22) of fibrosis on chest CT scan, 3 months after intensive care unit (ICU) discharge in SARS-CoV-2 survivors

    Truffaut L, Demey L, Bruyneel AV, Roman A, Alard S, De Vos N, Bruyneel M. Post-discharge critical COVID-19 lung function related to severity of radiologic lung involvement at admission. Respir Res. 2021 Jan 21;22(1):29. doi: 10.1186/s12931-021-01625-y. PMID: 33478527; PMCID: PMC7819622.
  - 2021 transbronchial cryobiopsy in N=10 severe SARS-CoV-2 postmortem
    - Now we can reflect on almost 2 years of pandemic, our knowledge grows concerning COVID-19 sequelae. Indeed, pulmonary fibrosis represents a common finding in advanced COVID-19 pneumonia.
    - Analyses of lung CT images have 100% sensitivity and 67% specificity to detect histopathological fibrosis (collagen extension)

    Ball L, Barisione E, Mastracci L, Campora M, Costa D, Robba C, Battaglini D, Micali M, Costantino F, Cittadini G, Patroniti N, Pelosi P, Fiocca R, Grillo F. Extension of Collagen Deposition in COVID-19 Post Mortem Lung Samples and Computed Tomography Analysis Findings. Int J Mol Sci. 2021 Jul 13;22(14):7498. doi: 10.3390/ijms22147498. PMID: 34299124; PMCID: PMC8305333.

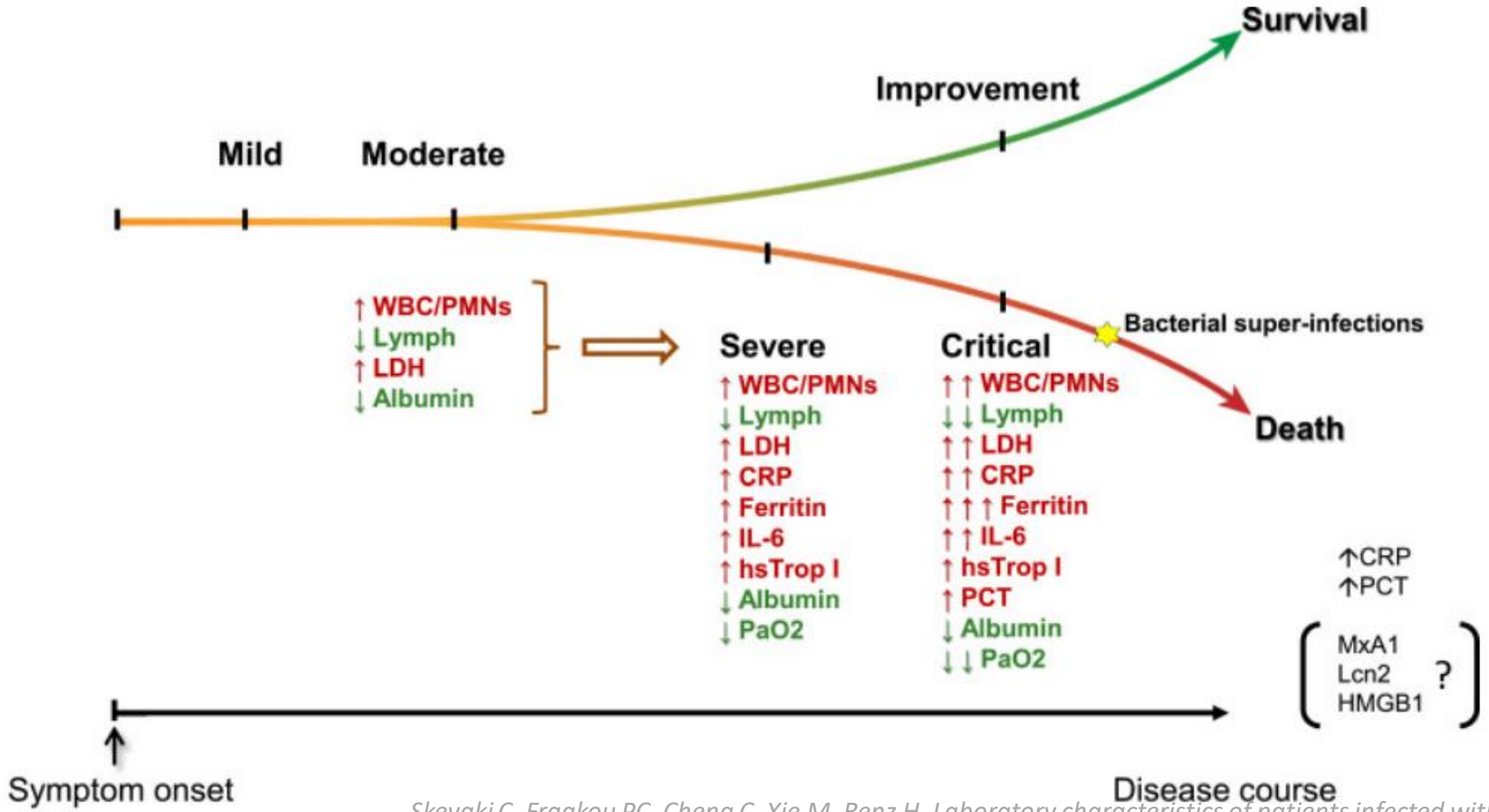
I. INTRODUCTION

BIOMARKERS OF INTEREST

- Classical biochemical markers reinforce the prognosis in COVID-19
  - CRP and Ferritin are induced by inflammatory cytokines, Ferritin is released by activated macrophages
  - Prognostic markers, associated with severity (CRP, ferritin), bacterial superinfection (CRP) and mortality (ferritin)
  - Extreme Ferritin increase is seen in Cytokine storm syndrome and secondary Hemophagocytic lymphohistiocytosis

Skevaki C, Fragkou PC, Cheng C, Xie M, Renz H. Laboratory characteristics of patients infected with the novel SARS-CoV-2 virus. J Infect. 2020 Aug;81(2):205-212. doi: 10.1016/j.jinf.2020.06.039. Epub 2020 Jun 21. PMID: 32579986; PMCID: PMC7306198.

I. INTRODUCTION



Skevaki C, Fragkou PC, Cheng C, Xie M, Renz H. Laboratory characteristics of patients infected with the novel SARS-CoV-2 virus. J Infect. 2020 Aug;81(2):205-212. doi: 10.1016/j.jinf.2020.06.039. Epub 2020 Jun 21. PMID: 32579986; PMCID: PMC7306198.

I. INTRODUCTION

BIOMARKERS OF INTEREST

- Krebs von den Lungen-6 (KL-6) in secondary pulmonary fibrosis post-COVID-19
  - Local cytopathic effect of SARS-CoV-2 on alveolar epithelial cells type 2 (AT2) damage the pulmonary interstitium and create AT2 hyperplasia which expresses higher levels of KL-6
  - 27.7% COVID-19 patients developed reversible and 11.8% patients developed irreversible pulmonary fibrosis, which can be monitored with KL-6

    Xue M, Zhang T, Chen H, Zeng Y, Lin R, Zhen Y, Li N, Huang Z, Hu H, Zhou L, Wang H, Zhang XD, Sun B. Krebs Von den Lungen-6 as a predictive indicator for the risk of secondary pulmonary fibrosis and its reversibility in COVID-19 patients. Int J Biol Sci. 2021 Apr 10;17(6):1565-1573. doi: 10.7150/ijbs.58825. PMID: 33907520; PMCID: PMC8071769.
  - optimum cut-off value in serum to evaluate COVID-19 severity
    - 371 U/mL (sens=85.7%; spec=96.6%)

      Awano N, Inomata M, Kuse N, Tone M, Takada K, Muto Y, Fujimoto K, Akagi Y, Mawatari M, Ueda A, Izumo T. Serum KL-6 level is a useful biomarker for evaluating the severity of coronavirus disease 2019. Respir Investig. 2020 Nov;58(6):440-447. doi: 10.1016/j.resinv.2020.07.004. Epub 2020 Aug 21. PMID: 32863199; PMCID: PMC7441928.
    - 407 U/mL (sens=83%; spec=89%)

      d'Alessandro M, Cameli P, Refini RM, Bergantini L, Alonzi V, Lanzarone N, Bennett D, Rana GD, Montagnani F, Scolletta S, Franchi F, Frediani B, Valente S, Mazzei MA, Bonella F, Bargagli E. Serum KL-6 concentrations as a novel biomarker of severe COVID-19. J Med Virol. 2020 Oct;92(10):2216-2220. doi: 10.1002/jmv.26087. Epub 2020 Jun 9. PMID: 32470148; PMCID: PMC7283867.

I. INTRODUCTION

BIOMARKERS OF INTEREST

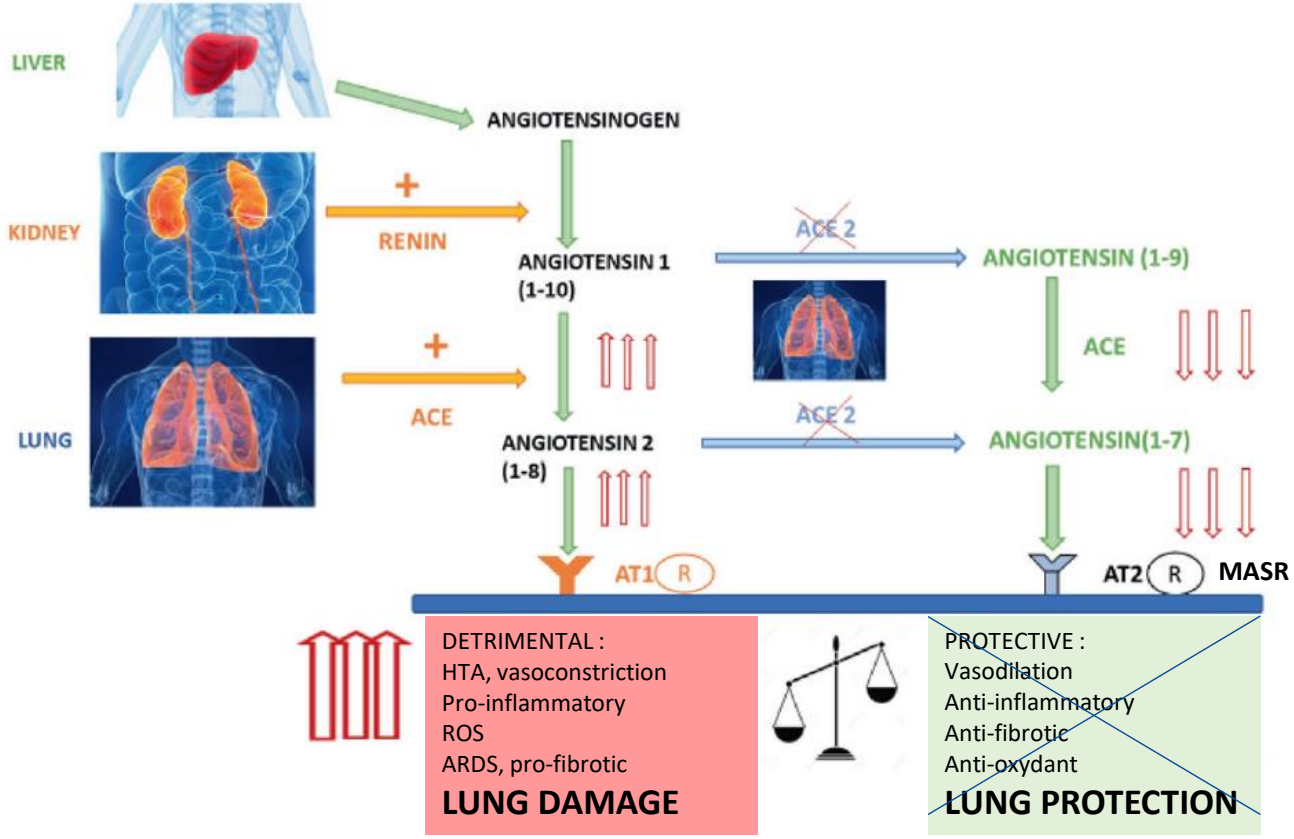
- Transforming growth factor beta (TGF-β) is “the master regulator of fibrosis”
  - Activation of the TGF-β pathway contributes to pathologic fibrosis in most organs by regulating fibroblasts’ transdifferentiation, enhances extra-cellular matrix protein synthesis through collagen type I and connective tissue growth factor gene promotion

    Ismaeel A, Kim JS, Kirk JS, Smith RS, Bohannon WT, Koutakis P. Role of Transforming Growth Factor-β in Skeletal Muscle Fibrosis: A Review. Int J Mol Sci. 2019 May 17;20(10):2446. doi: 10.3390/ijms20102446. PMID: 31108916; PMCID: PMC6566291.

  - In first 2 weeks of severe COVID-19 TGF-beta peaks → inhibit NK cells

    Witkowski M, Tizian C, Ferreira-Gomes M, Niemeyer D, Jones TC, Heinrich F, Frischbutter S, Angermair S, Hohnstein T, Mattiola I, Nawrath P, McEwen S, Zocche S, Viviano E, Heinz GA, Maurer M, Kölsch U, Chua RL, Aschman T, Meisel C, Radke J, Sawitzki B, Roehmel J, Allers K, Moos V, Schneider T, Hanitsch L, Mall MA, Conrad C, Radbruch H, Duerr CU, Trapani JA, Marcenaro E, Kallinich T, Corman VM, Kurth F, Sander LE, Drosten C, Treskatsch S, Durek P, Kruglov A, Radbruch A, Mashreghi MF, Diefenbach A. Untimely TGFβ responses in COVID-19 limit antiviral functions of NK cells. Nature. 2021 Dec;600(7888):295-301. doi: 10.1038/s41586-021-04142-6. Epub 2021 Oct 25. PMID: 34695836.

I. INTRODUCTION
axes AngII on AT1R versus Ang-1-7 on MAS



Adapted from Silhol F, Sarlon G, Deharo JC et al. Downregulation of ACE2 induces overstimulation of the renin-angiotensin system in COVID-19: should we block the renin-angiotensin system?. 2020 *Hypertens Res*. 43(8):854-856. DOI:10.1038/s41440-020-0476-3

I. INTRODUCTION

BIOMARKERS OF INTEREST

- ACE2 receptor for SARS-CoV-2 Spike protein and angiotensine pathway

Delpino MV, Quarleri J. SARS-CoV-2 Pathogenesis: Imbalance in the Renin-Angiotensin System Favors Lung Fibrosis. Front Cell Infect Microbiol. 2020 Jun 12;10:340. doi: 10.3389/fcimb.2020.00340. PMID: 32596170; PMCID: PMC7303284.

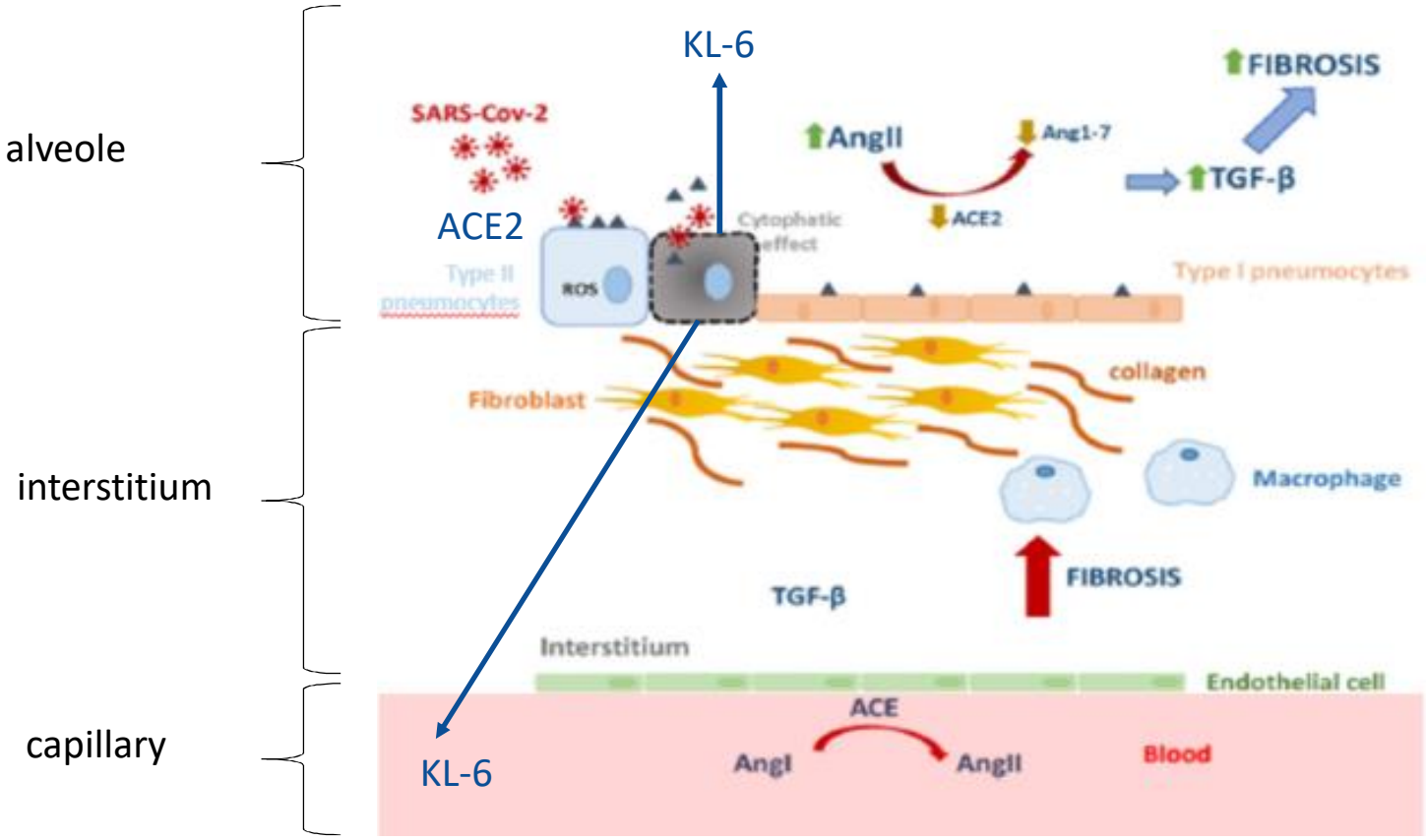
Li SR, Tang ZJ, Li ZH, Liu X. Searching therapeutic strategy of new coronavirus pneumonia from angiotensin-converting enzyme 2: the target of COVID-19 and SARS-CoV. Eur J Clin Microbiol Infect Dis. 2020 Jun;39(6):1021-1026. doi: 10.1007/s10096-020-03883-y. Epub 2020 Apr 13. PMID: 32285293; PMCID: PMC7152693.

  - ACE2 receptor is highly expressed in pneumocytes AT2.

  - Disequilibrium between pro-inflammatory/pro-fibrotic angiotensine II (AngII) axis and the counterbalancing angiotensine-1-7 (Ang-1-7) axis
    - Lung damage is caused by AngII (through the action of ACE) and leads to ARDS
    - AngII is metabolized into Ang-1-7 through the action of ACE2
    - Ang-1-7 protects the lung and binds to AT2R and Mas-receptor
    - ACE2 and Ang-1-7 are downregulated in severe COVID-19 and AngII predominates

  - Ang-1-7 could provide novel therapeutic interventions for pulmonary fibrosis patients
    - Candidate for theranostics

I. INTRODUCTION



Redrawn and modified from Delpino et al. 2020 Front Cell Infect Microbiol

CONTENT

I. INTRODUCTION

II. AIM

III. MATERIALS AND METHODS

IV. RESULTS

V. DISCUSSION & PERSPECTIVES

II. AIM

Prognostic role of biomarkers in pulmonary fibrosis post-COVID-19

1. Observe if Ang-1-7 diminishes and TGF-beta, KL-6, CRP, ferritin increase
   - In most severe COVID-19 patients at ICU
   - Kinetics will be monitored within-subject

2. Follow-up of survivors post-ICU for pulmonary sequelae
   - Clinical investigation and sample collection 3, 6, 12, 24 months in pulmonology department

3. Theranostics
   - Establishment of an algorithm where diagnostic tests drive therapeutic decisions

CONTENT

III. METHODS

- Pre-analytical phase
  - AngII is unstable : EDTA-tube on ice, immediate cold centrifugation, then -80°C
  - KL-6, TGF-beta, Ang-1-7 matrix comparison serum & heparin plasma (Wilcoxon)
  - Sample stability analysis 2-8°C, -20°C and -80°C

- Analytical performance study
  - Repeatability, reproducibility, bias, T.E., LOQ, linearity (Simple Regression Correlation)
  - Passing-Bablok correlation (if instrument available in another Belgian lab)
  - CE-IVD kits for AngII (RIA, DiaSource and ELISA RUO), KL-6 (ECLIA, Lumipulse, Fujirebio), ACE activity kit (Bülmann) on Cobas 8000 (Roche), CRP (immunoturbidimetry on Cobas c702 from Roche), ferritin (ECLIA on Cobas e801 from Roche)
  - ELISA RUO kits for ACE2, TGF-beta and Ang-1-7

III. METHODS

- Clinical study
  - Case description of each severely-ill COVID-19 patient at ICU (retrospective study) and during follow-up post-ICU (prospective study)
  - Biomarker kinetics in retrospective samples of severe COVID-19 patients at ICU
    - control group comparison
    - interpretation in function of symptomatology, disease severity and the classical biochemistry parameters

- Patient population
  - Retrospective clinical study: N=60 severe and critical COVID-19 patients at ICU in CHU Saint-Pierre (& Hôpital Erasme for KL-6 cut-off)
    - compare to groups of mild/asymptomatic COVID-19, healthy, and non-COVID-19 pneumonia
    - exclude patients R/chemo
  - Prospective observational study: N=60 COVID-19 survivors 3,6,12,24 months post-ICU in CHU Saint-Pierre & CHU Brugmann
    - compare to age-/sex-adjusted controls
  - Ethical committee approval CE/20-06-04 for 'Pulmonary recovery of ICU-COVID-19 survivors'

III. METHODS

- Statistical methods
  - GraphPad Prism, Analyse-it Excel
  - Comparison between groups use non-parametric tests (statistical significance $p<0,05$)
  - Multivariate analysis for prognosis of bad COVID-19 outcome (ICU-admission, mechanical ventilation, length of hospital stay, severity scores SOFA, APACHE, oxygenation index, radiological suspicion of pulmonary fibrotic lesions and death)
  - Statistical significant patient number (n=14)

$$n = \frac{2 \cdot (1.96+1.28)^2 \cdot \sigma^2}{\Delta m^2}$$

CONTENT

RESULTS OF PULMONARY EXPLORATION

Truffaut *et al. Respir Res* (2021) 22:29
https://doi.org/10.1186/s12931-021-01625-y

Respiratory Research

LETTER TO THE EDITOR Open Access

Post-discharge critical COVID-19 lung function related to severity of radiologic lung involvement at admission

Laurent Truffaut[1,6], Lucas Demey[1,6], Anne Violette Bruyneel[2], Alain Roman[3,6], Stephane Alard[4,6], Nathalie De Vos[5] and Marie Bruyneel[1,6*]

- CT scan
  - At ICU, patients show ground glass opacities (e.g. figure a) with a mean of 17/20 affected segments
  - 3 months post-ICU, N=19/22 show fibrosis (e.g. arrow figure b) with a mean of 8/20 affected segments
- Pulmonary function test
  - 3 months post-ICU, 45% abnormal DLCO (diffusing capacity of lungs for carbon monoxide)
- Evaluation of exercise capacity
  - 3 months post-ICU, 65% abnormal 6MWT (6-min walking distance test) with 30% of oxygen desaturation

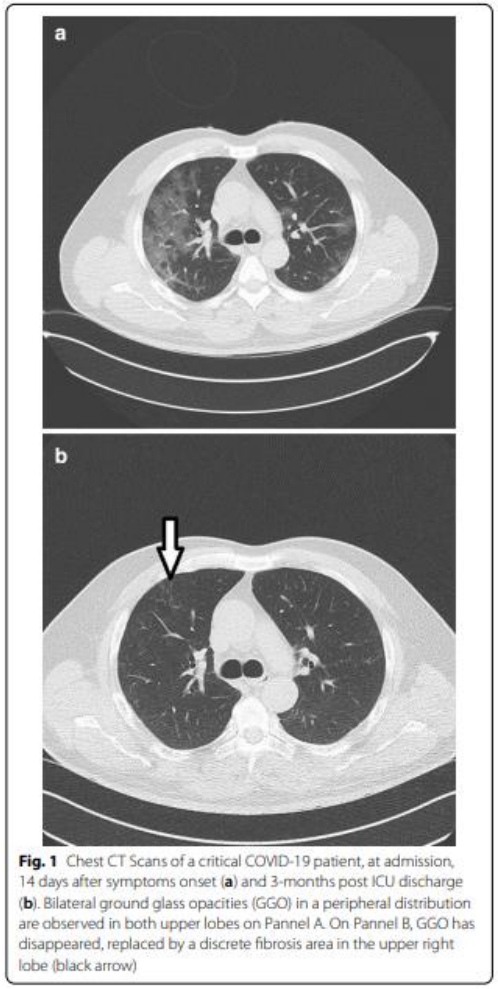


Fig. 1 Chest CT Scans of a critical COVID-19 patient, at admission, 14 days after symptoms onset (**a**) and 3-months post ICU discharge (**b**). Bilateral ground glass opacities (GGO) in a peripheral distribution are observed in both upper lobes on Pannel A. On Pannel B, GGO has disappeared, replaced by a discrete fibrosis area in the upper right lobe (black arrow)

RESULTS :
Analytical performance studies

- Analytical performance study for AngII : predefined performance criteria have not been met (nor for RIA, nor for ELISA)
- The polynomial quadratic function needed to calculate ACE2 did not give reliable results
- ACE insert

Performance criteria	ACE kinetic kit (Bühlmann®, Basel, Switzerland)
Repeatability	2,7 %
Intermediate precision	8,1%
Specificity	• Inhibition by EDTA, natural Angiotensine I
Measuring range	• Linearity : up to 108,9 U/L • LOQ : 12 U/L • LOD : <5 U/L
Interferences	Lipemic, hemolysed and icteric samples
Reference interval	Adults : 20-70 U/L 6 months - 18 years : 29 – 112 U/L 0 mois – 6 mois : very low values Sarcoïdosis : 45-135 U/L

RESULTS :
Analytical performance studies

ÅKL-6

Repetability	IQC L1 IQC L2	n=5	**CV = 6%** **CV = 5%**
Reproductibility	IQC L1 IQC L2	In duplicate, 10days	**CV = 7,2%** Bias = 7,9% TE = 19,6% **CV = 5,6%** Bias = 1,8% TE = 11%
LOD/LOQ	IQC L1	5-times serial dilution 1:2, triplicate	**14 U/mL** CV= 11%
Linearity	IQC L2	5-times serial dilution 1:2, triplicate	**y = 0,9966x - 21,298** **R^2 = 0,995** *Range = 26-816 U/mL*
Assay comparison	Sample	n=14	**y = 1,101x + 21,31** Intercept : -2,867 to 52,02 Slope : 0,9537 to 1,141
Matrix comparison	Serum vs heparin	n=10	*Wilcoxon test* ***p-value = 0,187*** No statistically significant difference
Specimen stability	Sample	n=10 (4°C) n=6 (-80°C) n=3 (freeze/thaw cycle)	*Wilcoxon test* **Stable : 7 days at 2-8°C** **14 days at -80°C** **3 freeze/thaw cycle**
Comparaison d'instrument *(Passing-Bablok, Bland-Altman)*	Echantillons patient	n=35	**y = 9,261 + 0,9301x** **Biais moyen : 3.65%** Ordonnée à l'origine : 1,41 to 25,54 Pente : 0,8952 to 0,9566

predefined performance criteria:
TE<20,8% achieved

LHUB-ULB versus ULg on G600II

G600II versus G1200

CLINICAL RESULTS of KL-6

POSTER : **ANALYTICAL PERFORMANCE STUDY OF THE NOVEL BIOMARKER KL-6 AND POSSIBLE INTEREST IN COVID-19**

Ponthieux F.[1], Lauwers M.[1], Duterme C.[1], Cavalier E.[2], Delattre I.[1], Deprez G.[1], Cotton F.[1], De Vos N.[1*]

The KL-6 Lumipulse® G test **reaches** the predefined acceptance criteria for analytical performance.
Preliminary results on COVID-19 patients revealed **significantly higher KL-6 levels in severe** versus non-severe patients. In the group of severely-ill COVID-19 patients with ARDS in ICU, we found no difference between survivors and patients who died.
The limitation of these findings is the small sample size.

1. Severity marker ?

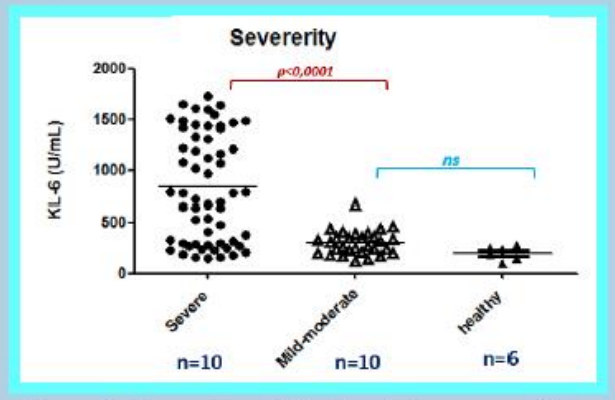


Figure 2 : Comparison of KL-6 levels in severe, mild-moderate COVID-19 patients and healthy controls.

Elevated KL-6 only in severe group with ARDS

2. Predictive of mortality ?

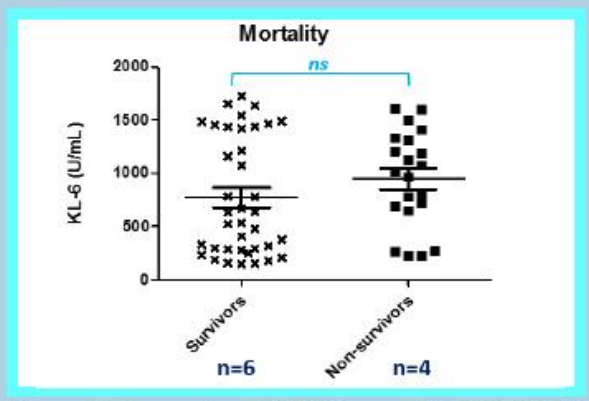
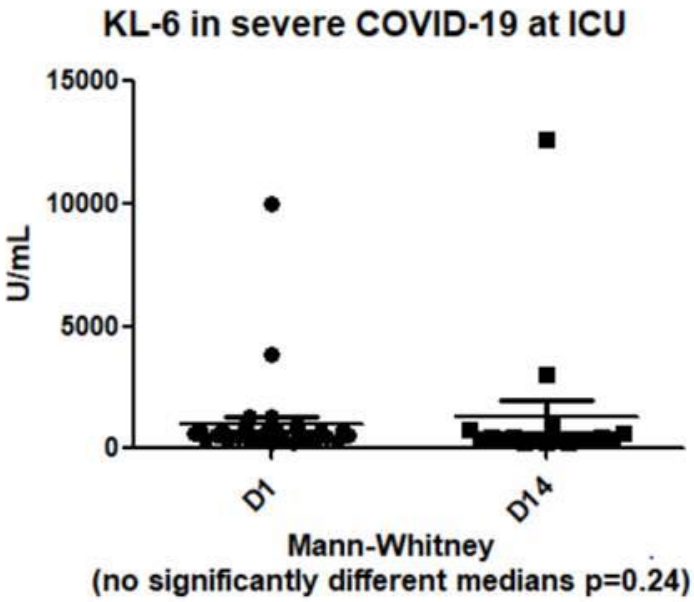


Figure 3 : Comparison of KL-6 in severely-ill COVID-19 survivors versus deceased patients

No significant difference in KL-6 levels between survivors and non-survivors

RESULTS

- No correlation between KL-6 and chemerin
- Levels were significantly higher in ICU patients than non-hospitalized patients :
  - KL-6 at D1 ($p<0.05$)
  - Chemerin at D1, D5, D14 ($p<0.0001$)
- No KL-6 kinetics during ICU stay



Chemerin plasma levels are increased in COVID-19 patients and are an independent risk factor of mortality, by Philomene Lavis, Sofia Morra, Carmen Orte Cano, Nurhan Albayrak, Véronique Corbière, Véronique Olislagers, Nicolas Dauby, Veronique Del Marmol, Arnaud Marchant, Christine Decaestecker, Francoise Mascart, ***Nathalie De Vos****, Philippe van de Borne, Isabelle Salmon, Myriam Remmelink, Marc Parmentier, Alessandra Kupper Cardozo, Benjamin Bondue, published in Frontiers in Immunology, section Cytokines and Soluble Mediators in Immunity*

RESULTS OF KL-6 FOLLOW-UP

Follow-up of KL-6 and routine biomarkers in severe COVID-19 one year post-ICU

Nathalie DE VOS [1*], Cécile DUTERME [1], Zahia OUANANI [1], Dominique DIRICKX [1], Marie BRUYNEEL [2], Alain ROMAN [3], Stephane ALARD [4], Fanny PONTHIEUX [1], Mailis LAUWERS [1], Elise MATHIEU [1], Cédrick GOUDJI [1], Stéphanie ANDRE [5], Dragos BARGLAZAN [1], Cécile DUSART [2], Laurent TRUFFAUT [2], Frédéric COTTON [1]

MATERIALS AND METHODS

Samples were prospectively drawn from 22 severe/critical COVID-19 patients during their pulmonology follow-up (FU) at 3, 6 and 12 months post-ICU.

RESULT 1) PATIENT CHARACTERISTICS

Patient demographic characteristics were: 73% men, mean age of 55 years, 50% obese, 45% arterial hypertension, 27% diabetes, 100% acute respiratory distress syndrome, a mean length of stay of 21 days at ICU, 64% with endotracheal intubation and 9% extracorporeal membrane oxygenation during the acute SARS-CoV-2 infection.

RESULTS OF KL-6 FOLLOW-UP

RESULT 2) BIOMARKERS AT ICU

At ICU, the severe acute COVID-19 patients showed significantly higher biomarkers LDH [503 U/L (404-747)], cTnT [20.15 ng/L (10.93-34.38)], ferritin [539 µg/L (133-1504)], PCT [0.30 µg/L (0.06-1.20)], KL-6 [665 U/mL (441-900)] and CRP [97 mg/L (32-132)] compared to healthy controls (Fig.1 and 2).

KL-6 (Fig.1) and CRP (Fig.2) marked the severity, as they were higher in severe acute COVID-19 patients at ICU compared to asymptomatic/mild acute COVID-19 cases.

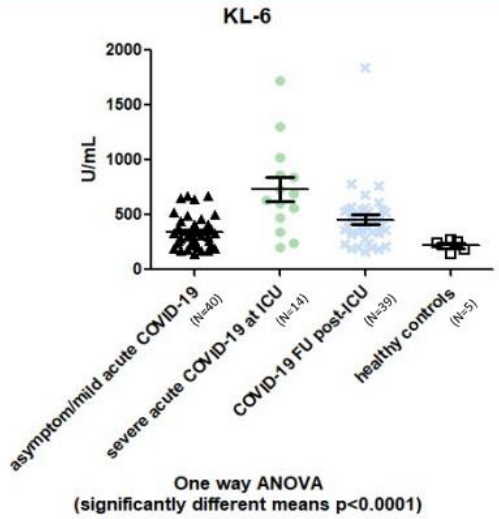


Fig 1. KL-6 in COVID-19 patients versus controls

Consecutive measurements were taken per patient. N represents the number of measuring points.

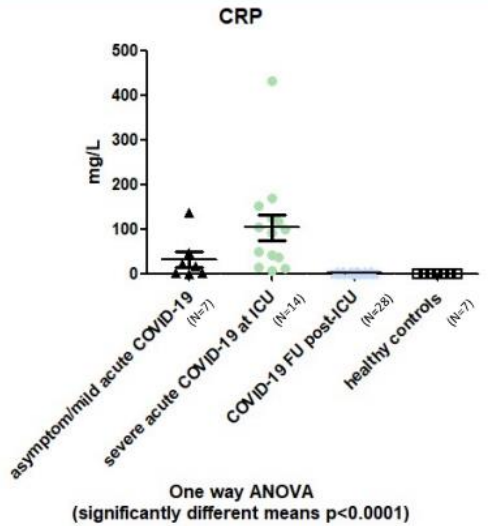


Fig 2. CRP in COVID-19 patients versus controls

Consecutive measurements were taken per patient. N represents the number of measuring points.

RESULTS OF KL-6 FOLLOW-UP

RESULT 3) FOLLOW-UP OF BIOMARKERS AT 3, 6 AND 12 MONTHS POST-ICU

Three months after ICU the biomarkers LDH, cTnT, ferritin, PCT and CRP turned normal in ≥90 % of patients. Figure 2 shows indeed normalization of CRP results in COVID-19 FU post-ICU.

After 1 year, KL-6 normalized (<275 U/mL) in only 25% of critical COVID-19 patients. Indeed, Figure 1 shows a significant different mean between COVID-19 FU post-ICU and healthy controls. The ROC curve of KL-6 levels has been determined to distinguish between severe COVID-19 (at ICU with ARDS) and healthy controls. Figure 3 shows a cut-off of 275 U/mL and AUC=0.929 (LR= ∞, SENS=86%, SPEC=100%, YI=0.857). Between 3,6 and 12 months of follow-up, the mean KL-6 value did not significantly change (Fig.4).

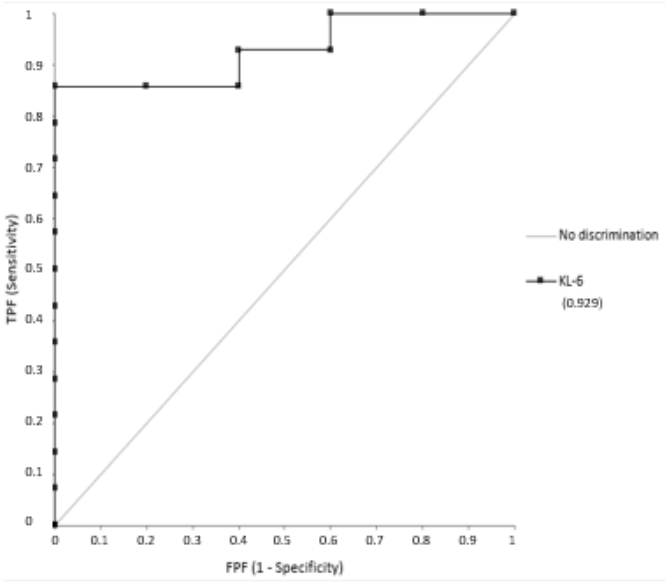


Fig 3. ROC curve of KL-6 levels

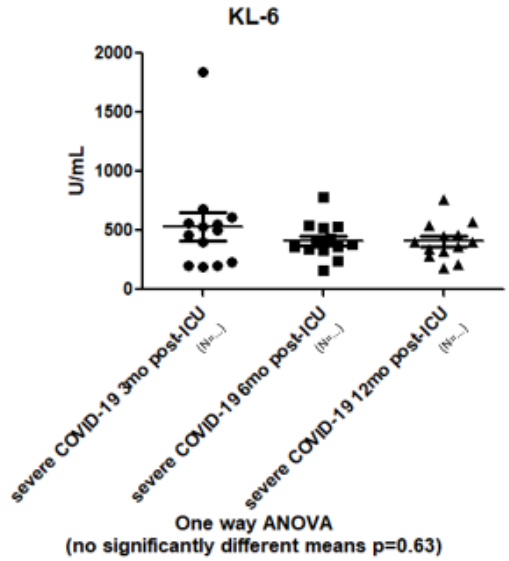


Fig 4. Follow-up of KL-6 after ICU discharge

RESULTS of KL-6 and ACE cut-offs

POSTER :

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CHU Saint-Pierre UMC Sint-Pieter

IFCC CRITICAL ROLE OF CLINICAL LABORATORIES IN THE COVID-19 PANDEMIC FEBRUARY 15-17, 2021

Novel biomarkers in critical COVID-19 patients with progression to pulmonary fibrosis

Nathalie DE VOS [1*], Fanny PONTHIEUX [1], Maïlis LAUWERS [1], Cécile DUTERME [1], Marie BRUYNEEL [2], Alain ROMAN [2], Stephane ALARD [1], Frédéric COTTON [1]

KL-6 CUT-OFF

ROC curve for KL-6 shows AUC=0.888 (Fig 3). Above the cut-off of 455 U/mL for KL-6, patients are very likely to belong to the group of severe/critical COVID-19 at ICU with ARDS (LR=20.6, SENS=71%, SPEC=97%, YI=0.675).

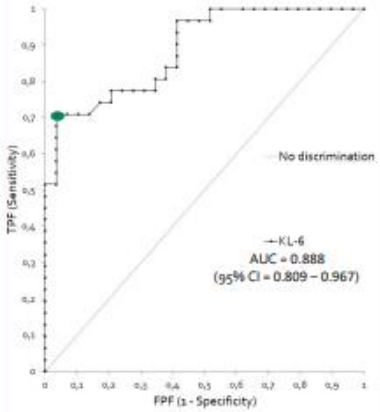


Fig 3. ROC curve of KL-6 levels

ACE CUT-OFF

ROC curve of ACE levels shows AUC=0.769 (Fig 4). Above the cut-off of 38 U/L for ACE, patients are more likely to belong to the group of severe/critical COVID-19 at ICU with ARDS (LR=3.5, SENS=64%, SPEC=82%, YI=0.455).
Another ROC curve built on the slope of ACE kinetics shows even better AUC of 0.954. Increasing ACE kinetics above the cut-off of 0.65 U/L/day, places patients in the group of severe/critical COVID-19 at ICU with ARDS with a very high probability (LR= ∞, SENS=80%, SPEC=100%, YI=0.800).

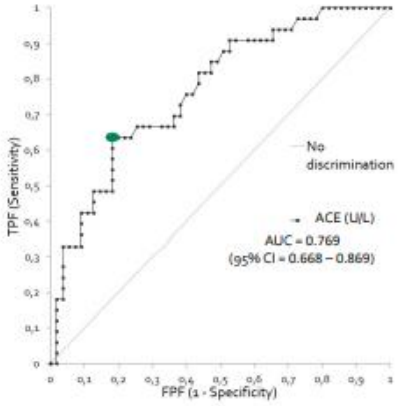


Fig 4. ROC curve of ACE levels

CLINICAL RESULTS OF ACE

RBSLM Annual Meeting 20/11/2020 :

C2 - Best Abstract in Clinical Chemistry

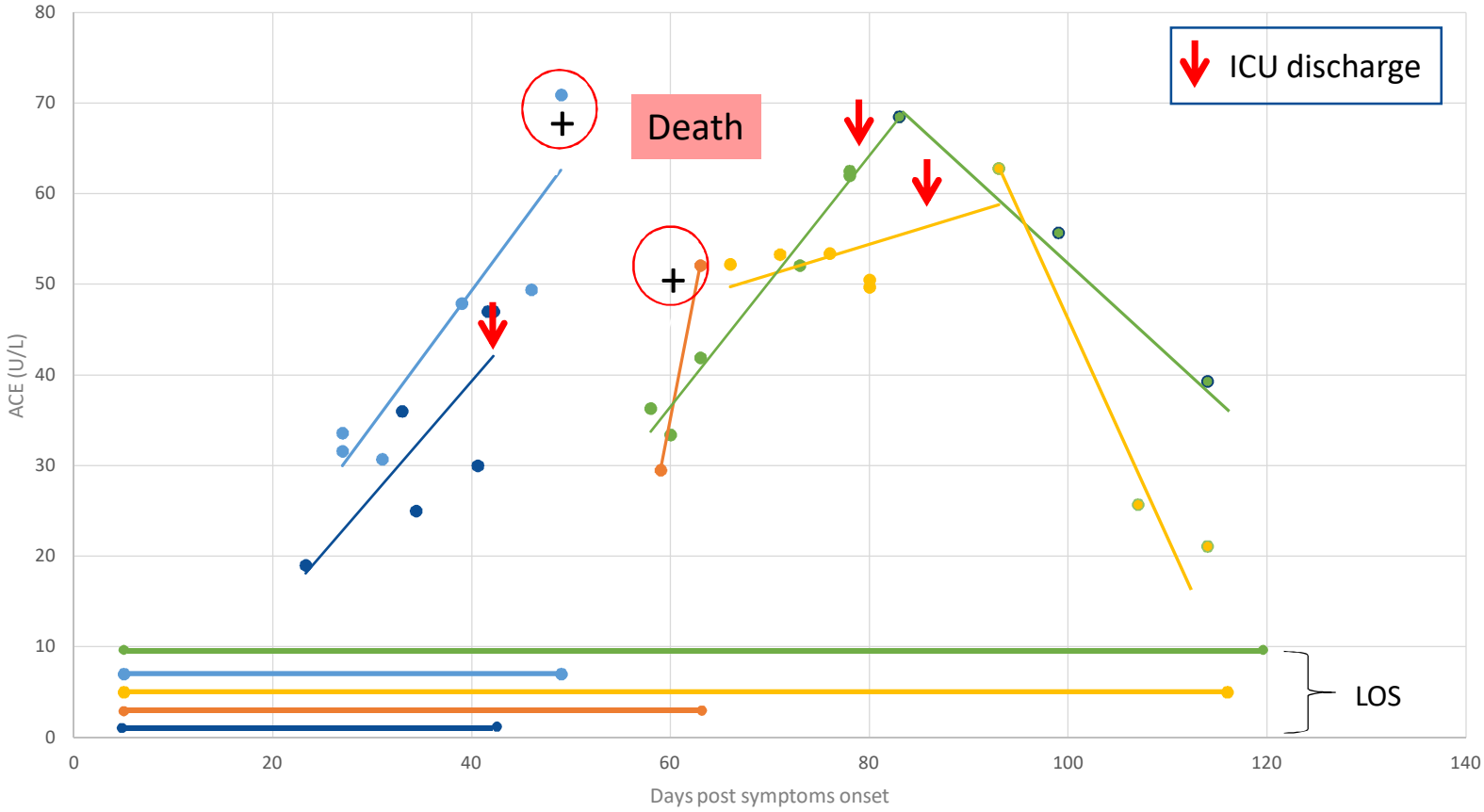
ASCENDING ACE KINETICS IN SEVERE COVID-19 PATIENTS AT ICU

M. Lauwers[1], S. Cherkaoui[1], F. Ponthieux[1], C. Duterme[1], I. Delattre[1], L. Seaux[1], M. Bruyneel[2], L. Truffault[2], A. Roman[3], G. Deprez[1], F. Cotton[1], N. De Vos[1]

- Severe COVID-19 pts at ICU had significant higher ACE values [median (range) = 50 (34-53) U/L] compared to mild/asymptomatic COVID-19 patients [median (range) = 35 (24-45) U/L; Mann-Whitney: p=0.0078)] and controls [median (range) = 28 (22-35) U/L; Mann Whitney: p=0.0014)].

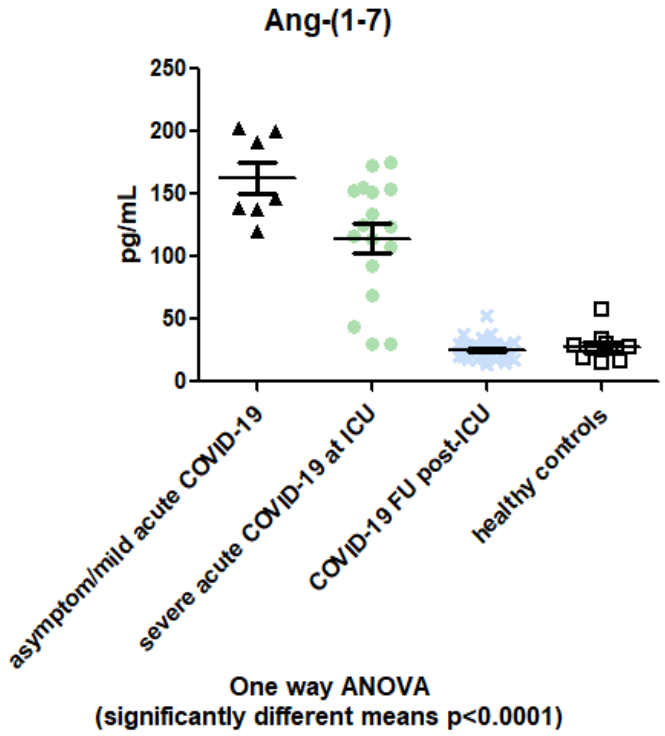
- In contrast to the control group, the severe COVID-19 patients clearly showed increasing ACE kinetics in consecutive samples. The 2 ICU-patients who died, had a steeper increase in ACE kinetics, with a maximum elevation of 5.65 U/L/day during the last 2 weeks of ICU-hospitalization.

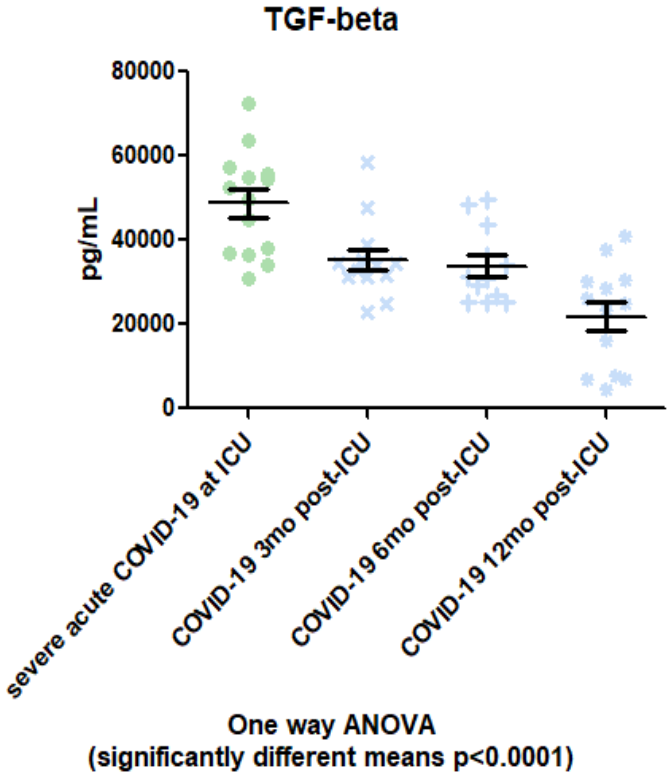
RESULTS of ACE



UNPUBLISHED RESULTS

Statistical significant different pattern in acute severe COVID-19:
Ang-1-7 diminishes and TGF-beta increases at ICU, with gradual decrease up to 1 year of FU



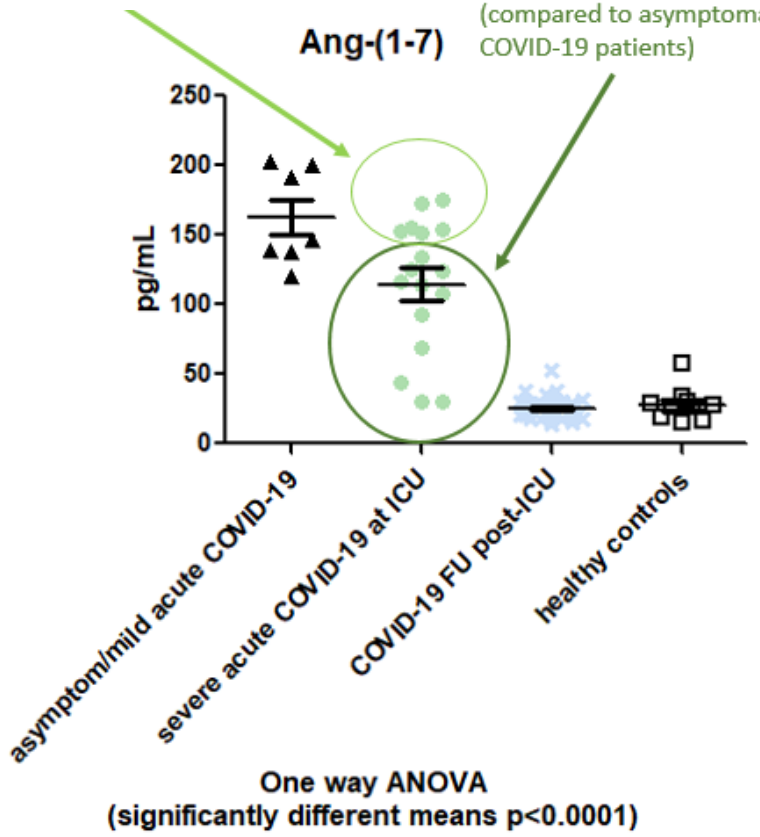


UNPUBLISHED RESULTS

Subpopulation at higher risk of COVID-19 pulmonary fibrosis/sequelae. This very ill subpopulation could be treated:

Diagnosis with Global composite index

Diagnosis with **lower** Ang-(1-7) levels (compared to asymptomatic/mild COVID-19 patients)



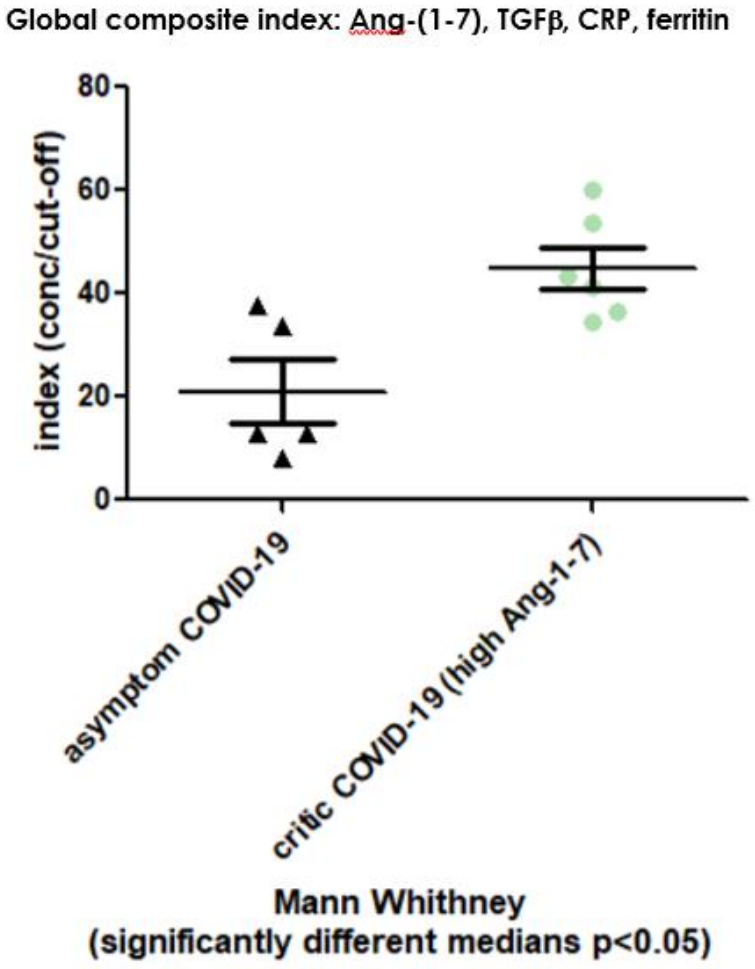
One way ANOVA
(significantly different means p<0.0001)

- **dexamethasone**
- **mechanical ventilation**
- **high oxygen flow**
- **antifibrotics** (pirfenidon, nintedanib)
- **medication in clinical trials:**

e.g. Ang-(1-7) agonist (patent EP2967049), recombinant human ACE2 (GlaxoSmithKline GSK2586881), MAS-receptor activator (Biophytis BIO101)

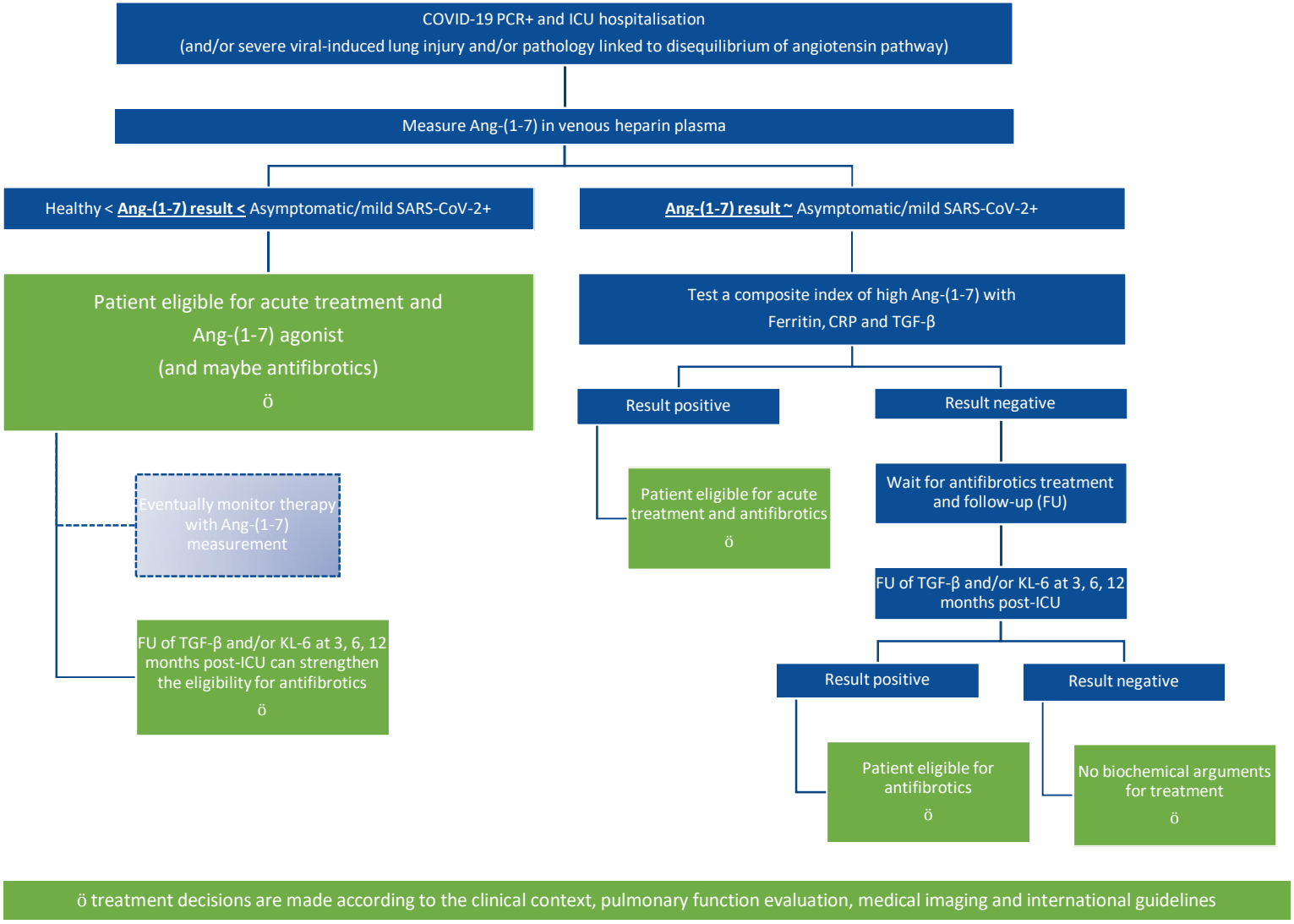
→ **Measure Ang-(1-7) to diagnose the critical subpopulation needing specific treatment**

UNPUBLISHED RESULTS



UNPUBLISHED RESULTS

Theranostics pathway for severe/critical COVID-19 patient



CONTENT

Discussion

1. Ang-1-7 diminishes and ACE, TGF-beta, KL-6, CRP significantly increase ($p<0,05$)

   - In most severe COVID-19 patients at ICU
   - At ICU there is within-subject kinetics for ACE but not for KL-6
   - KL-6 cut-off
     - ≥ 455 U/mL* to distinguish between critical COVID-19 and asymptomatic COVID-19

**Cut-off KL-6 = 406.5 U/mL according to d'Alessandro M, Cameli P, Refini RM, Bergantini L, Alonzi V, Lanzarone N, Bennett D, Rana GD, Montagnani F, Scolletta S, Franchi F, Frediani B, Valente S, Mazzei MA, Bonella F, Bargagli E. Serum KL-6 concentrations as a novel biomarker of severe COVID-19. J Med Virol. 2020 Oct;92(10):2216-2220. doi: 10.1002/jmv.26087. Epub 2020 Jun 9. PMID: 32470148; PMCID: PMC7283867.*

Discussion

2. Follow-up of survivors post-ICU for pulmonary sequelae
   - CT scans show pulmonary fibrosis already 3 months post-ICU in 86% of patients
   - Diffusing capacity of lungs for carbon monoxide is altered in half of the patients
   - Further research is needed to analyze if pulmonary sequelae are related to the level of some biomarkers
     - KL-6 persisted in 75% of critical COVID-19 survivors after 1 year
     - TGF-beta diminished gradually during the first year post-ICU

Discussion

3. Theranostics
   - Establishment of an algorithm where diagnostic tests drive therapeutic decisions
     - Ang-1-7 low, TGF-beta high, composite index high
       - Novel therapies for critical COVID-19 patients, currently in clinical trials
       - Anti-Spike Ab for mild COVID-19 patients without oxygen therapy but with a severity risk
   - WHO guideline for COVID-19 therapy contains the state-of-the art therapies

Recommended for patients with severe or critical COVID-19:

- a strong recommendation for systemic corticosteroids;
- a strong recommendation for interleukin-6 (IL-6) receptor blockers (tocilizumab or sarilumab), in combination with corticosteroids;
- a strong recommendation for baricitinib as an alternative to IL-6 receptor blockers, in combination with corticosteroids;
- a conditional recommendation for casirivimab-imdevimab for patients with seronegative status, where rapid viral genotyping is available and confirms infection with a susceptible SARS-CoV-2 variant.

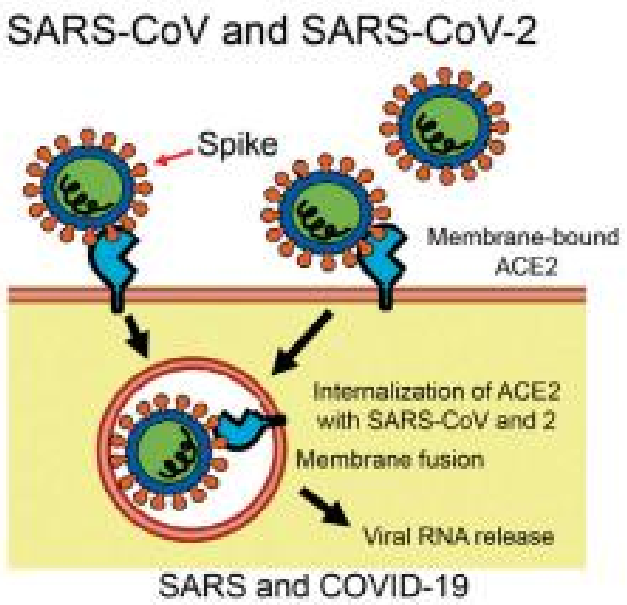
NOVEL THERAPEUTIC OPTIONS targeting Angiotensine pathway

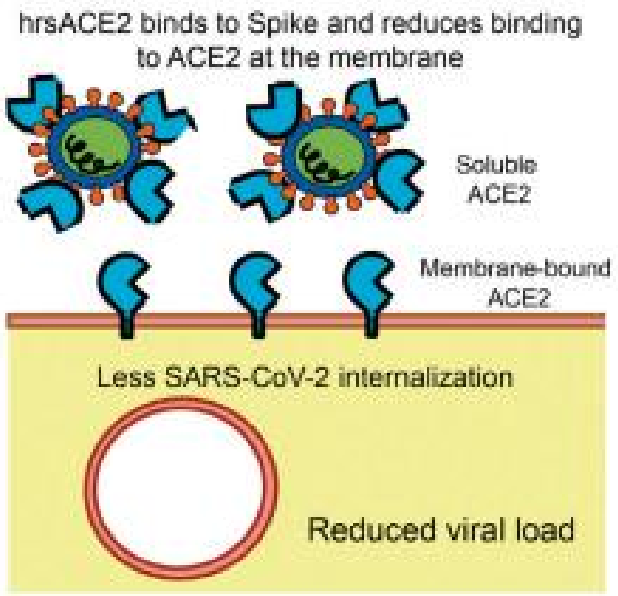
- Ang-1-7 agonist
  - Ang-1-7 supplementation
  - MAS-receptor agonist (BIO101)

Stimulation of the protective arm of the RAS with MasR agonists and activators				
Treatment of Angiotensin Peptide (1–7) for COVID-19	I	Standard of care plus Angiotensin peptide (1–7)-derived plasma vs. standard of care	Kanuni Sultan Suleyman Training and Research Hospital, Turkey	NCT04375124
TXA COVID-19 Clinical Trial	II	TXA127 vs. placebo	Columbia University, USA	NCT04401423
Angiotensin-(1,7) Treatment in COVID-19: the ATCO Trial	II/III	Angiotensin 1–7 vs. placebo	Erasme University Hospital Brussels, Belgium	NCT04332666
Testing the Efficacy and Safety of BIO101 for the Prevention of Respiratory Deterioration in COVID-19 Patients (COVA)	II/III	BIO101 vs. placebo	Biophytis, France	NCT04472728

Latil M, Camelo S, Veillet S, Lafont R, Dilda PJ. Developing new drugs that activate the protective arm of the renin-angiotensin system as a potential treatment for respiratory failure in COVID-19 patients. Drug Discov Today. 2021 May;26(5):1311-1318. doi: 10.1016/j.drudis.2021.02.010.

NOVEL THERAPEUTIC OPTIONS
hrsACE2 = pan-SARS-CoV-2 therapeutics





Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, Leopoldi A, Garreta E, Hurtado Del Pozo C, Prosper F, Romero JP, Wirnsberger G, Zhang H, Slutsky AS, Conder R, Montserrat N, Mirazimi A, Penninger JM. Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. Cell. 2020 May 14;181(4):905-913.e7. doi: 10.1016/j.cell.2020.04.004. Epub 2020 Apr 24. PMID: 32333836; PMCID: PMC7181998. https://doi.org/10.1016/j.drudis.2021.02.010.

NOVEL THERAPEUTIC OPTIONS targeting TGF-beta

- Antioxidants
  - **N-acetyl cysteine (NAC)** prevented Ang-II-induced expression of TGF-β1 and CTGF, suggesting that Ang-II-induced expression of the pro-fibrotic factors involves NOX-induced ROS. [1]
  - An isothiocyanate in **broccoli :** sulforaphane activates the transcription factor NF-E2-related factor 2 (Nrf2) [2]

- AT1R blockers & ACE inhibitors
  - Losartan blunts TGF-β signaling [3]
  - Enalapril does not reduce TGF-beta signaling pathway [4]

- Transcription factors: antifibrotic activity of **miR-29** in animal models by inhibiting TGF-beta and suppressing collagen expression
  - **micro noncoding regulatory RNA**, in the pathogenesis of fibrosis by regulating ECM production and deposition, and epithelial–mesenchymal transition [5]

1. *Morales M.G., Vazquez Y., Acuna M.J., Rivera J.C., Simon F., Salas J.D., Alvarez Ruf J., Brandan E., Cabello-Verrugio C. Angiotensin II-induced pro-fibrotic effects require p38MAPK activity and transforming growth factor beta 1 expression in skeletal muscle cells. Int. J. Biochem. Cell Biol. 2012;44:1993–2002. doi: 10.1016/j.biocel.2012.07.028.*

2. *Sun C., Li S., Li D. Sulforaphane mitigates muscle fibrosis in mdx mice via Nrf2-mediated inhibition of TGF-beta/Smad signaling. J. Appl. Physiol. (1985) 2016;120:377–390. doi: 10.1152/japplphysiol.00721.2015.*

3. *Burks T.N., Andres-Mateos E., Marx R., Mejias R., Van Erp C., Simmers J.L., Walston J.D., Ward C.W., Cohn R.D. Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. Sci. Transl. Med. 2011;3:82ra37. doi: 10.1126/scitranslmed.3002227.*

4. *Morales M.G., Cabrera D., Cespedes C., Vio C.P., Vazquez Y., Brandan E., Cabello-Verrugio C. Inhibition of the angiotensin-converting enzyme decreases skeletal muscle fibrosis in dystrophic mice by a diminution in the expression and activity of connective tissue growth factor (CTGF/CCN-2) Cell Tissue Res. 2013;353:173–187. doi: 10.1007/s00441-013-1642-6.*

5. *Cushing L, Kuang P, Lü J. The role of miR-29 in pulmonary fibrosis. Biochem Cell Biol. 2015 Apr;93(2):109-18. doi: 10.1139/bcb-2014-0095. Epub 2014 Sep 18. PMID: 25454218.*

Healthcare economy

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Perspectives

- Further investigations on more patients are necessary to confirm the results
- Match with age and sex-related controls
- Relate the biomarker results to CT scan and pulmonary function tests
- Draft articles

Articles in the pipeline

with Prof. Frédéric Cotton and Prof. Marie Bruyneel

ÅClinical

1. Similar pulmonary functional outcomes at 3 months in critical COVID-19 survivors hospitalized during the first, second, and third pandemic waves
2. Multicentric clinical evolution 1 year post-ICU
3. Image of air trapping when dyspnea

ÅAnalytical

1. KL-6 is a novel biomarker for damaged alveolar type II pneumocytes, also in COVID-19
2. Theranostics in COVID-19 with novel biomarkers Ang-(1-7), TGF-beta and composite index
3. Biomarkers in COVID-19 one year post-ICU linked to clinical outcome

Merci pour votre attention

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Questions?

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