Myocardial Virus and Gene Expression in SARS-CoV-2 Positive Patients with Clinically Important Myocardial Dysfunction

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#AHA20
Disclosures

• President & CEO of a biotech company (ARCA biopharma) developing a drug for COVID-19 Coagulopathy (CAC)
  – Drug (rNAPc2) and indication have no direct relationship to this presentation or research program
Angiotensin II Formation in the Intact Human Heart
Predominance of the Angiotensin-converting Enzyme Pathway

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Angiotensin-(1-7) Formation in the Intact Human Heart
In Vivo Dependence on Angiotensin II as Substrate

Lawrence S. Zisman, MD; Glenn E. Meixell, PhD; Michael R. Bristow, MD, PhD; Charles C. Canver, MD
Circulation. 2003;108:1679-1681
October 7, 2003

Increased Angiotensin-(1-7)–Forming Activity in Failing Human Heart Ventricles
Evidence for Upregulation of the Angiotensin-Converting Enzyme Homologue ACE2

Lawrence S. Zisman, MD; Rebecca S. Keller, PhD; Barbara Weaver, MS; Qishan Lin, PhD; Robert Speth, PhD; Michael R. Bristow, MD, PhD; Charles C. Canver, MD
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C.S. Sampling
HPLC peptide separation
The Renin-Angiotensin-Aldosterone System

Substrate or Effector
- Enzyme
- Receptor
- Inhibitor

Angiotensinogen
- Renin
- ACE
- Angiotensin I

Angiotensin-(1-7)
- NEP
- MLN-4760
- ACE2

Angiotensin II
- ACE
- Angiotensin I

Aldosterone
- Mineralocorticoid R

Mineralocorticoid R

(Vasodilation, anti-inflammatory, anti-hypertrophic)

ACE inhibitors

ARBs

AT₁ R
AT₂ R

MRAs

β₁-AR blockers
renin inhibitors

Bristow MR et al, JBTS Sept 2020. DOI: 10.1016/j.jacbts.2020.06.007; Published online June 25, 2020
Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus

Wenhui Li, Michael J. Moore, Natalya Vasilieva, Jianhua Su, Swee Kee Wong, Michael A. Berne, Mohan Somasundaran, John L. Sullivan, Katherine Luzuriaga, Thomas C. Greenough, Hyeryn Choe & Michael Farzan

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A pneumonia outbreak associated with a new coronavirus of probable bat origin

Peng Zhou, Xing-Lou Yang, Xian-Guang Wang, Ben He, Lei Zhang, Wei Zhang, Hua-Yu Li, Yan Zhu, Bei Li, Xiao-Lin Huang, Hai-Dong Chen, Jing Chen, Yan Luo, Hua Guo, Ren-Di Jiang, Mei-Qin Liu, Ying Chen, Xu-Rui Shen, Xi Wang, Xiao-Shuang Zheng, Ke Zhai, Quan-Xiao Chen, Fei Deng, Lin-Lin Liu, Bing Yan, Fa Xian Zhan, Yan-Yi Wang, Guo-Fu Xiao & Zhong-Li Shi

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Since the outbreak of severe acute respiratory syndrome (SARS) 18 years ago, a large number of SARS-related coronaviruses (SARSr-CoVs) have been discovered in their natural reservoir host, bats. Previous studies have shown that some bat SARSr-CoVs have the potential to infect humans. Here we report the identification and characterization of a new coronavirus (2019-nCoV), which caused an epidemic of acute respiratory syndrome in humans in Wuhan, China. The epidemic, which started on 12 December 2019, had caused 2,794 laboratory-confirmed infections including 80 deaths by 26 January 2020. Full-length genome sequences were obtained from five patients at an early stage of the outbreak. The sequences are almost identical and share 79.6% sequence identity to SARS-CoV. Furthermore, we show that 2019-nCoV is 96% identical to the whole-genome level to a bat coronavirus. Pairwise protein sequence analysis of seven conserved non-structural proteins domains show that this virus belongs to the species of SARS-CoV. In addition, 2019-nCoV virus isolated from the bronchoalveolar lavage fluid of a critically ill patient could be neutralized by sera from several patients. Notably, we confirmed that 2019-nCoV uses the same cell entry receptor—angiotensin converting enzyme II (ACE2)—as SARS-CoV.
As of 4/6/20 (AHA grant submission):

• Myocardial injury & dysfunction minimal reports, mechanism uncertain
  – Myopericarditis by CMR (1 report, no tissue)
  – 1 heart autopsy biopsy, SCD in severe lung Dz, “no obvious histologic changes” in heart

• Myocardial injury evidence by ↑ hs-cTn, associated with adverse outcomes

• ACE2 is CoV-2 receptor for cell entry, ↑ in failing/remodeled human LVs and in animal models Rxd with ARBs
Myocardial Virus and Gene Expression in SARS-CoV-2 Positive Patients with Clinically Important Myocardial Dysfunction: Aims

Aim 1. Detection of CoV-2 in cardiac myocytes.
- N = 10, EMBx
- Histopathology including EM, patients with evidence of CoV-2 myocardial involvement
- RT-PCR for viral genome

Aim 2. Determine the degree of inflammatory reaction vs. direct myocardial injury.
- Histopathology
- Cytokine gene expression, circulating levels

Aim 3. Measure mRNA expression of the binding target (ACE2), proteases and integrins that have been shown to be key to cellular entry in non-cardiac cells.
- mRNA abundance by RNA-Seq and microarray
- ACE2, ACE, NPPB, α5 ITG, TF, mRNA abundance by RT-PCR rapid turnaround; circulating ACE2, ACE, ANG II, TF

Aim 4. Measure mRNA expression of candidate and global genes, and compare results to nonfailing controls and reduced LVEF nonischemic dilated cardiomyopathy (NDC) patients
- mRNA abundance by RNA-Seq and microarray, n = 10 patients with CoV-2 myocardial involvement
- 12 NF, 12 F/NDC septal biopsies from explanted hearts; previous EmBx data (4 NF, 46 F/NDC)
Myocardial Virus and Gene Expression in SARS-CoV-2 Positive Patients with Clinically Important Myocardial Dysfunction: Revised Entry Criteria (10/1/20)

Inclusion, Hospitalized Patients

- In or recently in ICU, PCR + for CoV-2, Age ≥18, COVID-19 myocardial involvement in the DDx, stable enough for cardiac catheterization
- LVEF <50% OR
  - TnI ≥0.05 ng/ml OR
  - Global longitudinal strain > -16 OR
  - ST-T changes suggesting STEMI, NSTEMI or myopericarditis with patent coronary arteries OR new onset sustained VT or VF
- Patient or authorized representative able to give informed consent

Inclusion, Outpatients

- In ICU in the past 3 mos, PCR + for CoV-2, Age ≥18, COVID-19 myocardial involvement in the DDx, stable enough for cardiac catheterization
- LVEF, TnI, GLS, ST-T and VT/VF criteria same as for hospitalized patients
- Patient able to give informed consent
Dynamic Regulation of SARS-CoV-2 Binding and Cell Entry Mechanisms in Remodeled Human Ventricular Myocardium

**Highlights:**

1. Cellular receptor for CoV-2 (ACE2) and 5 proteases previously implicated in membrane fusion are expressed.
2. ACE2 upregulated ≈2 fold in remodeled LV, proteases NSC.
3. ACE2 normalizes on reverse remodeling independent of ACEIs or ARBs.
4. ITGA5, which encodes an integrin (α5 ITG) that binds to ACE2 and to a motif (RGD) in the CoV-2 spike protein receptor binding domain, is upregulated in remodeled LV and normalizes on reverse remodeling, and is a candidate for facilitating or mediating CoV-2 cell binding and entry.

Thus upregulated CoV-2 cell binding mechanisms may explain heightened risk of COVID-19 in patients with underlying heart muscle disease.

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Bristow MR et al, JBTS Sept 2020. DOI: 10.1016/j.jacbts.2020.06.007; Published online June 25, 2020
Integrin $\alpha 5\beta 1$ facilitates CoV-2 binding and cell entry

ATN-161 (non RGD peptide derived from fibronectin, binds to $\alpha 5\beta 1$ at an $\alpha 5$ ITG binding site). In VeroE6 cells:
- CoV-2 binds to $\alpha 5\beta 1$
- ATN-161 prevents CoV-2 binding to $\alpha 5\beta 1$, (nM affinity), and $\alpha 5$ binding to ACE2 (μM)
- ATN-161 prevents CoV-2 cell infection (3.16 μM IC<sub>50</sub>)

Bristow MR et al, JBTS Sept 2020. DOI: 10.1016/j.jacbts.2020.06.007
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Myocardial Involvement in COVID-19: Summary so far

- Clinically significant myocardial involvement in COVID-19 patients occurs with uncertain but not uncommon incidence, and is important to detect and monitor following the acute infection.

- Myocardial injury, most commonly detected by an elevation in hs-cTn, may be of several types:
  - Inflammation (myocarditis); probably over Dx’d based on uncontrolled CMR studies.
  - Cytopathic effects in cardiac myocytes including myofibril disruption and loss, with no or little evidence of inflammation.
  - Vascular involvement, including microthrombi.

- ACE2 is upregulated in ventricular remodeling similar to NPPB, doesn’t appear to be modifiable by RASi therapy and may be a major reason for worse outcomes in some patients.

- Integrin α5 or its α5β1 dimer is a co-receptor for CoV-2, and is a potential therapeutic target in COVID-19.