"Long-COVID neurologico": disturbo psichico o malattia organica?

Contributo della neuropatologia alla comprensione del fenomeno

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Effects of SARS-CoV-2 on the Nervous System

Since the onset of the pandemic, neurological complications have emerged that have aggravated COVID-19, representing the basis for neurological long-COVID

Beside lung failure, neurological manifestations: headache, loss of smell, encephalopathy, confusion, stroke, encephalitis and post-infectious complication (acute disseminated or necrotizing/hemorrhagic encephalomyelitis, Guillain-Barré syndrome)
These studies were conducted in Lombardy, the epicentre of SARS-CoV-2 infection carrying 46% of the Italian cases, during the peak of the first COVID-19 outbreak.

Three-quarters of deaths occurring in the 70-79 (28.1%) and 80-89 (40.8%) age groups*

Also, the elderly with dementia affected by COVID-19 present almost 3 times higher mortality

(Hariyanto TI ‘20)

How SARS-CoV-2 causes encephalopathy: cytokines’ storm (IL6, IL1β, TNF) or brain infection? What about social isolation, and what is the impact on dementia?

(Mao L ’20; Zubair AS ‘20)

* Istituto Superiore di Sanità. Characteristics of COVID-19 patients dying in Italy report based on available data on May 7th, 2020
THREE DIFFERENT RESEARCHES TO ASSESS THE IMPACT OF COVID-19 ON AGING BRAINS

✓ Study 1: Retrospective analysis of COVID-19 cases with dementia, conducted during the spring of 2020, to clarify the incidence and prognostic significance of delirium.
✓ Study 2: Neuropathological and transcriptomic comparison between COVID-19 cases (with and without dementia) and matched non-COVID controls.
✓ Study 3: Telephone survey during lockdown, conducted on 204 cognitively assessed elderly.
Dizziness, lethargy, and psychomotor retardation may be part of the “Sickness Behavior“: a nonspecific cytokine-induced syndrome due to the activation of innate immunity

(Dantzer R ’02; Holmes C ’11)

Delirium may develop at the onset of COVID-19 and may be caused by specific underlying encephalitis. What are its prognostic implications?

(Kennedy M ’20; Poloni TE ’20)
Retrospective analysis of the medical records of 59 subjects with dementia and COVID-19, conducted during the pandemic peak (March 27-April 18, 2020) in a dedicated facility, in order to identify the prevalence and prognostic significance of delirium as an onset symptom of COVID-19.

57/59 (97%): SARS-CoV-2-positive (mean age: 82)
All had comorbidities: 32% with 3 or more diseases
Lymphopenia was a constant in these subjects.
The Overall mortality rate was 25%; dementia severity, by itself, had no impact on short-term mortality.
<table>
<thead>
<tr>
<th>Delirium – CAM</th>
<th>Delirium-onset COVID-19</th>
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<tbody>
<tr>
<td>Acute and fluctuating course</td>
<td>21 (100-0)</td>
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<tr>
<td>Inattention</td>
<td>21 (100-0)</td>
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<tr>
<td>Disorganized thinking</td>
<td>12 (57-1)</td>
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<td>Altered level of consciousness</td>
<td>19 (90-5)</td>
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<td>Lethargic</td>
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<td>Psychomotor agitation / anxiety</td>
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<td>Incessant talking/constant complaining</td>
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<tr>
<td>Wandering / Intrusiveness</td>
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<td>Physical and verbal aggression</td>
<td>8 (38-1)</td>
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<tr>
<td>Oppositional behaviour</td>
<td>18 (85-7)</td>
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<tr>
<td>Apathy / Inertness</td>
<td>10 (47-6)</td>
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<tr>
<td>Anorexia</td>
<td>12 (57-1)</td>
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<td>Resistance to care</td>
<td>10 (47-6)</td>
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<td>Psychotic symptoms</td>
<td>4 (19-0)</td>
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<tr>
<td>Hallucinations</td>
<td>1 (4-8)</td>
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<tr>
<td>Delusions</td>
<td>4 (19-0)</td>
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</table>
1. Delirium (hypoactive or hyperactive) represented the initial manifestation of COVID-19 in 21/57 (36.8%) patients. Delirium onset was strongly associated with mortality (p<0.001) and is independently associated with mortality risk (OR: 17.0; 95% CI: 2.8–102.7; p=0.002).

1. Sudden behavioral changes in the presence of lymphopenia deserve special attention. The pathological confirmation of virus invasion and a detailed characterization and topography of inflammatory infiltrate are needed to correctly interpret the mechanisms underlying delirium in COVID-19.
What Does Neuropathology Show?

**Vascular injuries**, both ischemic or haemorrhagic, including macroscopic and microscopic lesions due to clotting alterations, endotheliitis or sudden blood pressure changes in critically ill patients.

**Inflammation**, including ADEM-like features and different pictures of immune-induced meningoencephalitis with a variety of meningeal-parenchymal and perivascular lympho-monocytic infiltrates, and areas of microglial hyperactivation.
Immune hyperactivation involving the innate immune systems inside the brain (namely microglia) appears as a key factor in the pathogenesis of neurological damage, particularly in elderly individuals affected by neurodegenerative phenomena recruiting inflammation.

What happens in the brains of the elderly affected by COVID-19?

To describe COVID-19 neuropathology in elderly, and to verify where and how SARS-CoV-2 affects the inflammatory response in the brain and whether the inflammatory changes are due to COVID-19 or pre-existent neuropathology.

AIM

Serrano-Pozo '13; Santos '16; Hansen '18; Felsky '19
Neuropathological comparison between 9 COVID-19 cases (with and without dementia) and 6 matched non-COVID controls. The brains were analyzed by immunohistochemistry for SARS-CoV-2, lymphocytes, astrocytes, endothelium and microglia (semi-quantitative scale for microglial activation). In all cases, Alzheimer's disease (Thal-Braak) and the presence of small vessel disease (SVD) were assessed.
Abbiateggrosso Brain Bank protocol (partially modified), Poloni & Medici, Jove ‘20

For histology: 1 cm sections (fixed)

For ddPCR: 1 frontal sample from each COVID-19 case (frozen)

Stainings and reactions: H&E, LFB; 4G8, AT8; CD68, CD20, CD3; GFAP, CD34; anti-SARS-CoV-2
H&E reveals diffuse cortical oedema (a), inflammatory perivascular infiltrates (b), and micro nodules in the basal ganglia (c) and hippocampus (d), which are also identified with CD68 marker (e). LFB shows myelin loss in the subcortical WM due to SVD (f). GFAP reveals mild to moderate gliosis with frequent reactive astrocytes (g, g’). GFAP enhancement in astrocytic endfeet around blood vessels is a peculiar picture (h). CD34 staining displays foci of abnormal tuft-like capillary features, particularly frequent in the pons (i, j). Rare foci of perivascular T-lymphocytes (k) and B-lymphocytes (l) are identified using anti-CD3 and anti-CD20 antibodies, respectively. B-lymphocytes are also observed within some inflammatory nodules (m). Rare SARS-Cov-2 positive cells are detected only in the lower brainstem of a single case (n, o).
Comparison of the frontal cortex (a-d) and the subiculum (e-h) of 2 AD cases (one AD/COVID-19 and one AD/non-COVID) and a non-AD/non-COVID case. The AD/COVID-19 case shows amyloid and neuritic plaques (a, e), as well as moderate to severe microglial activation and nodules in a topographical distribution similar to that of the plaques (b, f). This microglial activation does not appear different from that seen in the AD/non-COVID case (c, g), while the same areas in the non-AD/non-COVID case are clearly diverse, with a normal microglial representation (d, h).

Different pictures of activated microglia (CD68+) in COVID-19 cases, including amoeboid cells, perivascular infiltrates and nodules in the olfactory bulb (i), frontal cortex (j), hippocampus (l, m), midbrain (n: red nucleus; o: detail of a nodule with neuronophagia in the upper right sector), pons (p: locus coeruleus q and r: raphe nuclei), and medulla oblongata (s: area close to DMV).
Microglial activation and nodules in the the **pons of a young asymptomatic SARS-CoV-2 positive man who died in an accident**. Inflammatory features are detectable in the pons and, to a lesser extent, in some white matter areas of the frontal lobe. Due to the absence of coexisting neurodegenerative lesions, neuroinflammation is not present in the brain cortex: **microglial reaction is case-specific and related to the personal history**
## Table 2 Microglial grading

Notes: COVID-19 cases are labeled as ‘COV’ and control cases are identified as ‘BB’ (for Brain Bank)

Legend: $+$ = present; $*$ = hyperactive delirium; (+) = previous episodes of delirium; $-$ = absent

**Micronodule grading:**
- ![none](none) = 0/none;  ![mild](mild) = 1/mild;  ![moderate](moderate) = 2/moderate;  ![severe](severe) = 3/severe

Abbreviations (in alphabetical order): AD, Alzheimer’s Disease; AD PATH, AD pathology; B, Braak stage; COV, COVID-19; NCD, neurocognitive disorder; DEL, delirium; GM, Gray matter; HC, hippocampus; Int, intermediate; MB, midbrain; MO, Medulla oblongata; n/a, not available; P, pons; SEP, sepsis; T, Thai stage; WM, white matter
Microglial activation in COVID-19

A. Microglial grading in COVID-19 and non-COVID cases per area

B. A significant difference is present only considering the whole brainstem

Legend: Small circles and stars represent out values and far out values; FCGM, frontal cortical gray matter; FWM, frontal white matter; HC, hippocampus; MB, midbrain; MO, medulla oblongata
Microglial activation in the frontal cortex considering the COVID-19 and dementia groups

Comparison of average microglial grading in the frontal cortex of COVID-19 cases, with or without Alzheimer’s dementia (AD), and of non-COVID cases with and without AD

Cortical microglial activation is increased by Alzheimer’s neuropathology more than by COVID-19

Indeed, in AD cases, COVID-19 determines only a slight and non-significant boosting of cortical microglial activation
Scatter plot showing **strong correlation between microglial activation** (in frontal cortex and hippocampus) **and amyloid-β/TAU pathology** in all 15 cases
In COVID-19 cases complicated by delirium there is significantly greater microglial activation in the hippocampus.

Through a tragic "natural experiment", SARS-CoV-2 is helping shed some light on the neuropathology underlying behavioral symptoms and delirium, as signs of limbic system dysfunction, which seem to be related to both degenerative load and microglial boosting in the hippocampus.

Although not significant, there is an association trend between the precocity of delirium and the severity of Braak stage.
Analysis of the frozen fronto-basal cortex of the COVID-19 cases in search of the SARS-CoV-2 genome, by means of qRT-PCR and ddPCR; in addition, a comparison with non-COVID cortex was made using "RNA-seq analysis" to highlight the transcriptional signature of COVID-19 cases.

Detection of SARS-CoV-2 genome and whole transcriptome sequencing in frontal cortex of COVID-19 patients

Stella Gagliardi a, Emanuele Tino Poloni b, Cecilia Pandini a, Maria Garofalo a,c, Francesca Dragoni a,c, Valentina Medici b, Annalisa Davin b, Silvia Damiana Visonà d, Matteo Moretti d, Daisy Sproviero a, Orietta Pansaras a, Antonio Gualta b, Mauro Ceroni e,f, Livio Tronconi g,h, Cristina Cereda h,i

Viral RNA in the cortex: minimal with qRT-PCR (1/9); detectable with ddPCR in 8/9: (88% of cases)

COVID-19 transcriptional signature: decreased HIF (hypoxia inducing factor, cellular oxygen modulator), increased hemoglobin subunits (HBB, A1, and A2) and lncRNA CTB-36O1.7 (microglial modulator)
The hallmarks of COVID-19 neuropathology

**HYPOXIA**

- Brain hypoxia → Vasodilation → Hyperemia → Edema
- Lungs infected with SARS-CoV-2
- Damaged alveoli → Decrease in oxygen exchange
- Hypoxemic blood

**NEURO-INFLAMMATION**
The hallmarks of “COVID-19 encephalitis”

- Higher in cases with both Alzheimer’s pathology and COVID-19, cortical inflammatory changes are not only related to COVID-19 but also to pre-existing neurodegeneration.
- Few viral traces (RNA and antigens) and scant lymphocytes argue against the presence of encephalitis and active viral replication within the CNS, and they do not suggest a neurotropism of SARS-CoV-2.
- COVID-19 brains showed microglial reinforcement with a low number of lymphocytes – enhancement of innate immunity and suppression of adaptive immunity.
The immunohistochemical detection of viral proteins is very scant in CNS and limited to isolate neurons in the lower brainstem: antigen migration through lower cranial nerves.

Viral RNA in the fronto-basal cortex is minimal and probably from blood.

TMPRSS2 and ACE2 expression is generally low into the brain.

Iadecola C. ’20

Does SARS-CoV-2 enter and persist into the brain?
COVID-19 encephalopathic syndrome

Acute changes in behavior (hyperactive or hypoactive delirium) and vegetative alterations (hypotension, hypersomnia, lack of "hypoxic drive"). This syndrome is well explained by the location of the inflammatory lesions, not surprisingly observed in the hippocampus and brainstem:

1. COVID-19 patients with NCD and delirium show a microglial boosting in the hippocampus, as a part of the **limbic system** – microglial activation associated with TAU-pathology

2. The brainstem area is affected by microglial hyperactivation in all COVID-19 cases (regardless of the cognitive state) – specific topographical phenomenon: inflammatory involvement of the brainstem and vegetative centers
Telephone survey, during the lockdown, on 204 cognitively assessed elderly: 164 normal (NOLD), 24 mild disorders (MCI), 18 mild-moderate dementia; psycho-socio-behavioral variables were analyzed through logistic regression to ascertain their effects on mood and memory.
1. Subjects with dementia were less adaptive, less active and more depressed;
2. NOLDs remained physically and mentally active but were more bored and anxious.
3. Lockdown increased risk of insomnia (OR: 10.26; CI: 1.13–93.41; p=0.039)
4. Insomnia independently increased the likelihood of depression (OR: 2.29; CI: 1.06–4.93; p 0.034)
5. Regular exercise was protective (OR: 0.30; CI: 0.12–0.72; p=0.007)
6. Worsening of memory disorders was associated with dementia (p=0.006).
After the pandemic...

The emerging threat of COVID-19 neuro-psychiatry

Probably, COVID-19 cases with dementia appears more prone to a strong and early microglial response, responsible for the “COVID-19 encephalopathy” which is more likely and severe in older people with NCD due to underlying chronic brain hypoxia, degenerative burden, “inflammaging” phenomena and “immunological senescence”.

On the other hand, the elderly with dementia suffered more from lockdown effects
Unsurprisingly, many older adults continue to experience symptoms after the acute phase of COVID-19 (long-COVID), especially of the neuropsychiatric type. The results clearly suggest that long-COVID in elderly patients is not the result of the persistence of SARS-CoV-2 within the brain but rather derives from a complex interaction between biological and psychosocial detrimental factors.

From this perspective, long-COVID can be interpreted as the consequence of COVID-19 encephalopathy. The damage to the brain parenchyma, and therefore the long-term consequences, will be serious in proportion to the severity of the acute phase encephalopathy.
Many thanks to my amazing women's team and to my forensic colleagues. Thanks for listening today.