Il Centro di Ricerca

FONDAZIONE GOLGI CENCI
RICERCHE E STUDI PER GLI ANZIANI

Neuropatologia
Brain Bank

Biologia
genetica

Ricerca sociale,
psicologica
Epidemiologia

Studio “InveCe.Ab” nella popolazione 70-75enne di Abbiategrasso: 2009 – 2014

Emanuele Poloni & Antonio Guaita

Roma, 15 novembre 2018

Guaita et al. BMC Geriatrics 2013, 13:98
http://www.biomedcentral.com/1471-2318/13/98

BMC Geriatrics

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Comune di Abbiategrasso

Asp Golgi Redaelli

A.S.L. MILANO 1

STUDY PROTOCOL Open Access

Brain aging and dementia during the transition from late adulthood to old age: design and methodology of the "Invece.Ab" population-based study

Antonio Guaita[1*], Mauro Colombo[2], Roberta Vaccaro[1], Silvia Fossi[1], Silvia Francesca Vitali[2], Gianluigi Forloni[3], Letizia Polito[1], Annalisa Davin[1], Virginia Valeria Ferretti[4] and Simona Villani[4]

InveCe.Ab

(ClinicalTrials.gov, NCT01345110)

NOLD-Mild-Major NCD trajectory

Obiettivo principale:

Prevalenza e incidenza di demenza e di deficit cognitivo in tutti i residenti nati dal 1935 al 1939

Obiettivo secondario:

Influenza delle variabili sociali, mediche, biologiche, psicologiche

Valutazione *(3h30'):*

Prelievo per parametri ematochimici e DNA (ApoE e polimorfismi)

Questionario sociale (variabili demografiche, attività, abitudini anche alimentari)

Misure antropometriche e test del cammino

Visita medica generale e neurologica

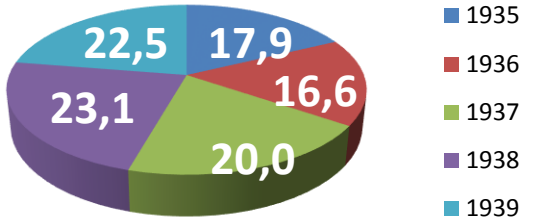
Colloquio e valutazione neuropsicologica (test per tutti i 6 domini cognitivi)

La popolazione dello studio InveCe.Ab:
1773 elegibili al baseline

2010

1321

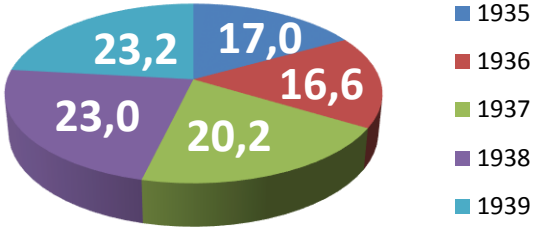
607 Uomini – 714 Donne



2012

1114

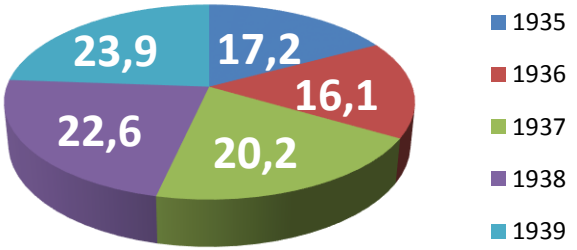
514 Uomini – 600 Donne



2014

1010

460 Uomini – 550 Donne



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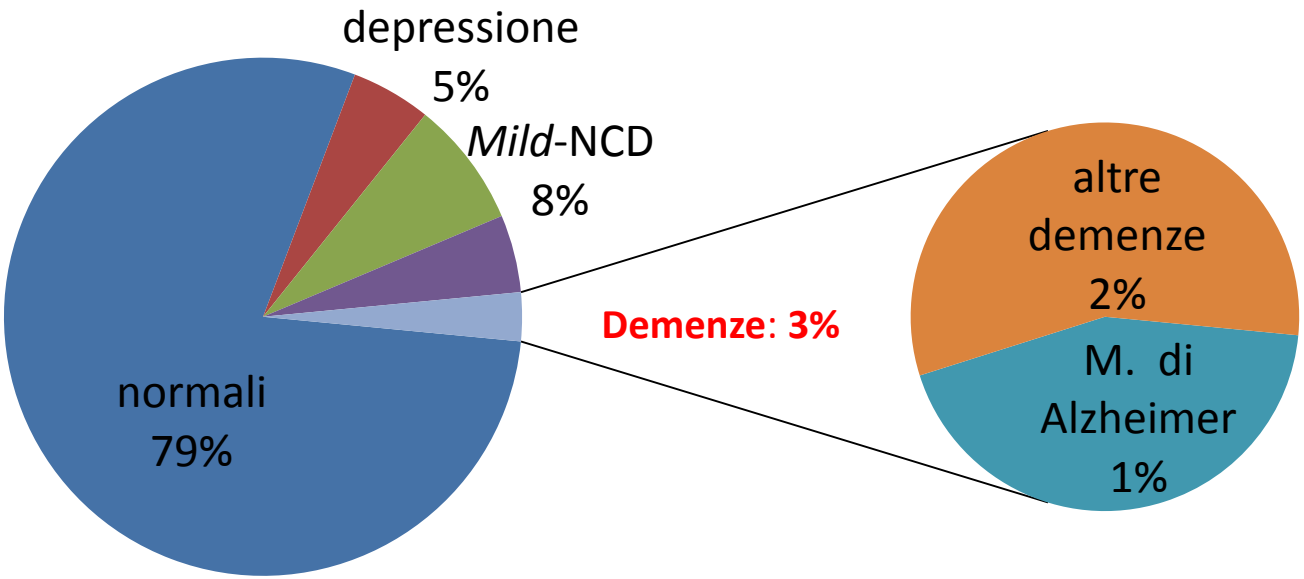
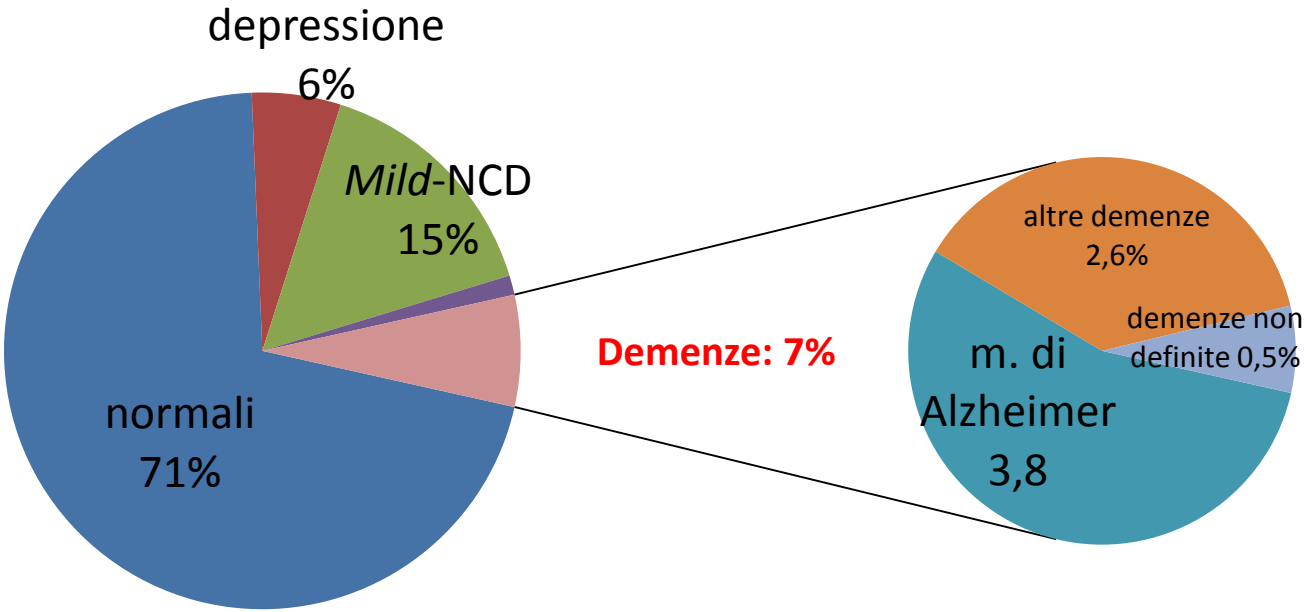


2010



2014

2018: ?

InveCe2 in corso

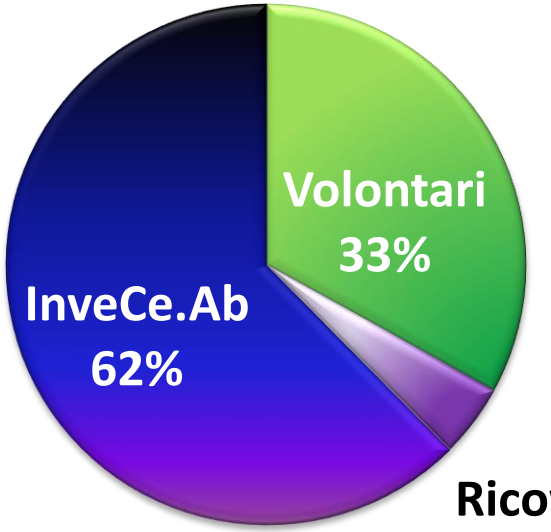
ISCRITTI ALLA BANCA (295)

Come diventare un donatore

- Consenso al follow-up
- Consenso alla donazione
- Consenso per il trattamento dei dati

Follow-up biennale dopo i 75 anni:

- Esame sangue
- Elettrocardiogramma
- Test neuropsicologici
- Elettroencefalogramma (qEEG)
- Visita neurologica



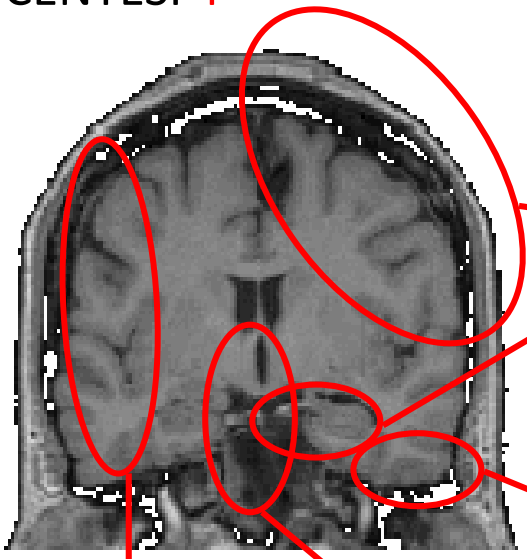
Diagnosi cliniche degli iscritti alla Banca (DSM V): NOLD 90% – *mild*-NCD 4% – *major*-NCD 6%; un terzo dei NOLD ha qualche disturbo del SN centrale o periferico, senza chiaro deficit cognitivo. Gli iscritti coprono una fascia di età tra i 62 e i 105 anni.

QUALE EZIOLOGIA PROBABILE? ESAMI AVANZATI PER LA **DETERMINAZIONE DI BIOMARCATORI**

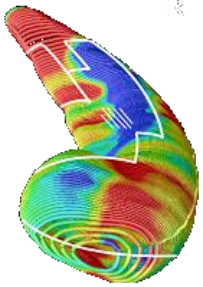
BIO-MARKER

RM 3 T 22,

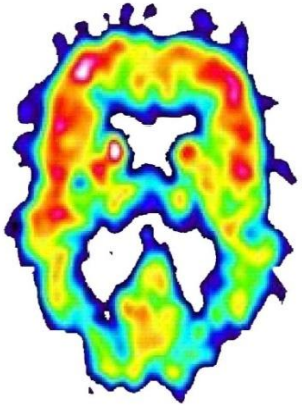
RACHICENTESI 4

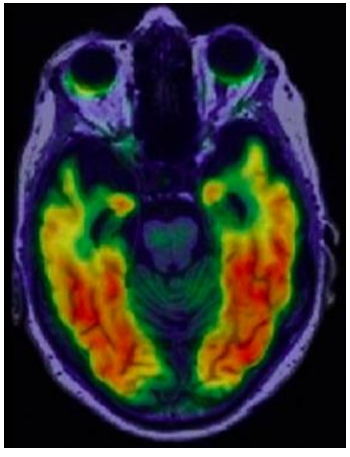


Structure:
MR hippocampal volumetry



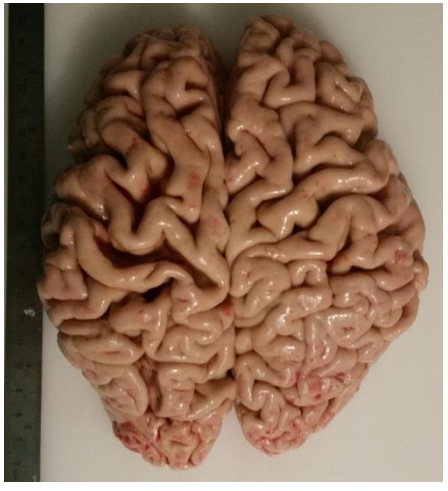
Metabolism:
FDG PET

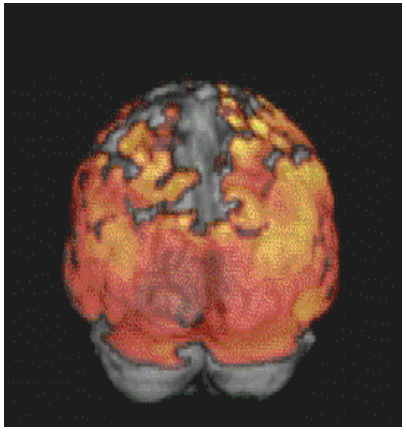




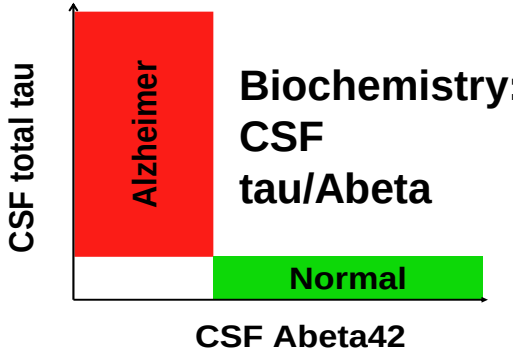
TAU pathology:
TAU PET

Neuropathological confirmation



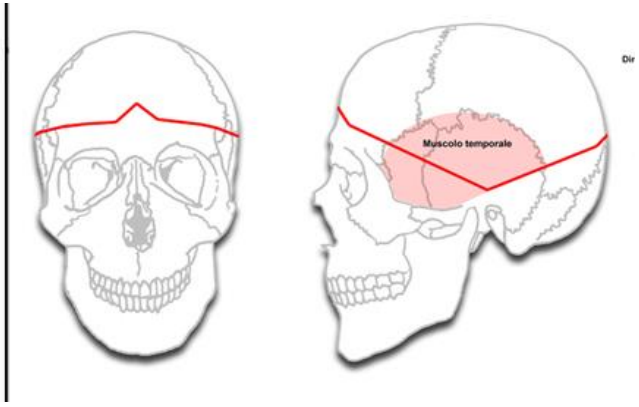


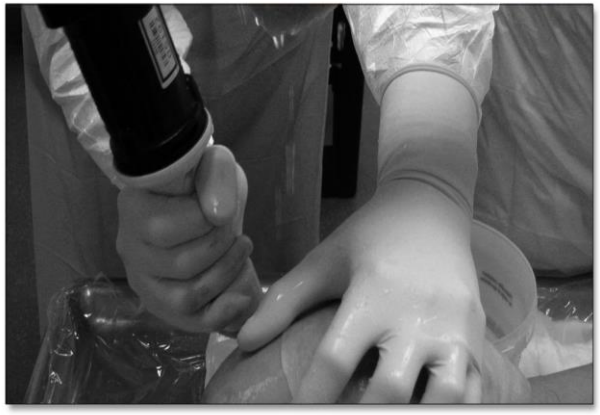
Amyloid deposits:
PIB PET



Biochemistry:
CSF tau/Abeta

Golgi-Cenci protocol for dissection and diagnosis

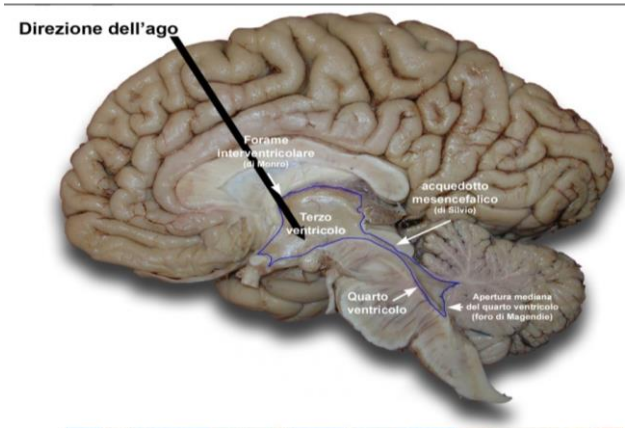




In neuropathological data base:

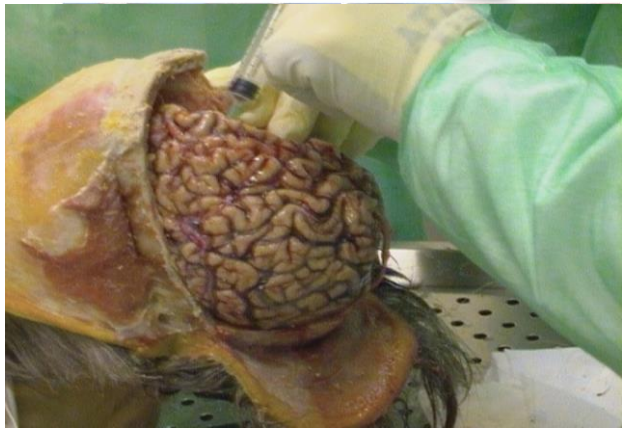
- Detailed neurological examination and clinical history
- Extensive NPS evaluation
- DSM V diagnosis
- CDR score before death
- Main concurrent diseases
- Cause of death and agonal state (AFS: 0-2)

The goal is to characterize and store good quality tissues (fixed and frozen) coming from subjects with a detailed longitudinal observation (aging trajectory)



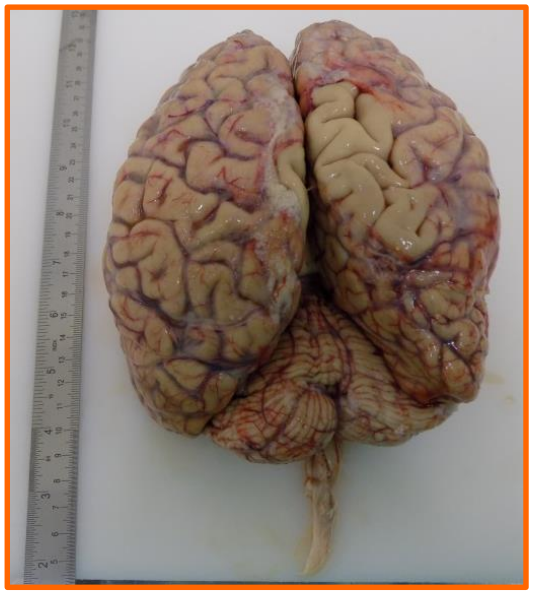
At demise the corpse undergoes tanatography and brain harvesting within 24 h. We have 20 brains collected, with a mean post-mortem interval of 10 hours

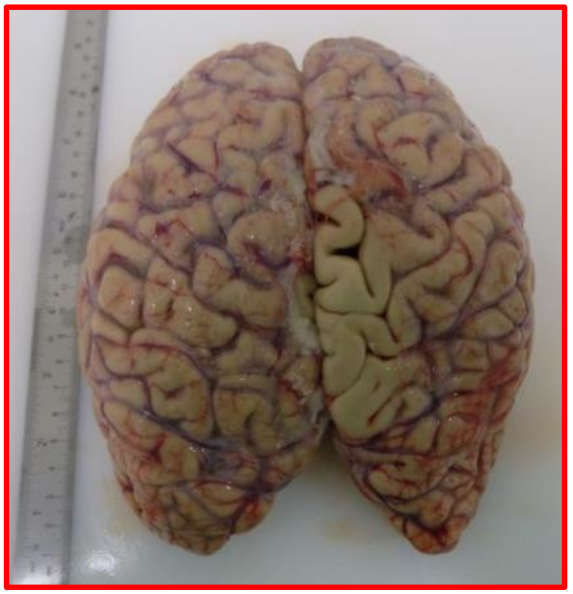
CSF pH -
(mean pH: 6.6)
Hemispheric pH -
(mean pH: 6.2)

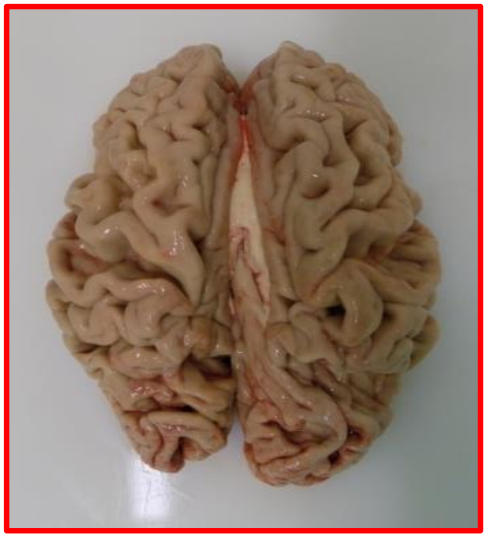


MACROSCOPIC EXAMINATION and PARTITION

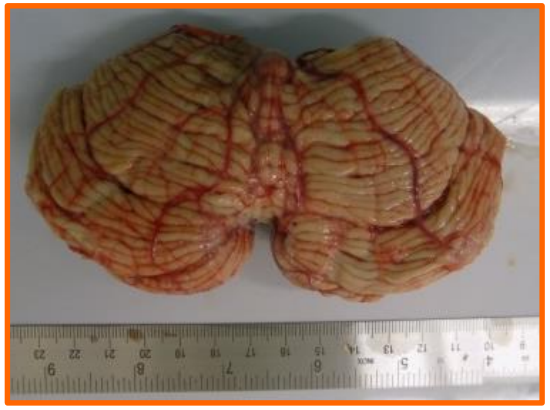
1. Macroscopic analysis (weight, atrophy, meninges, …)

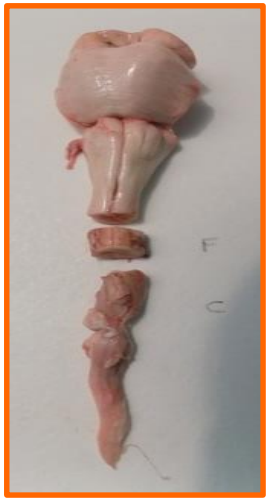






2. Partition of brain – cerebellum – brainstem – Willis circle





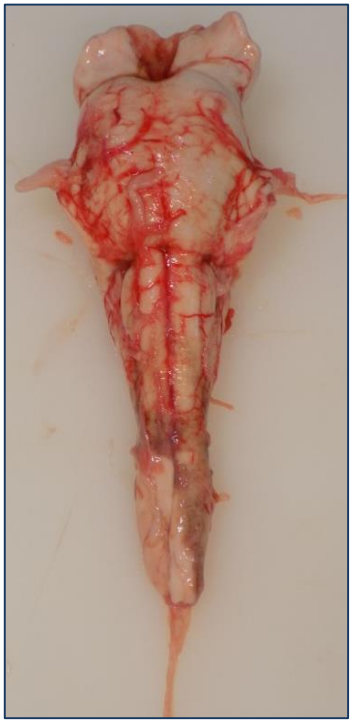
Esiri's grading (0 – 3)

We harvest leptomeninges samples for fibroblast colture

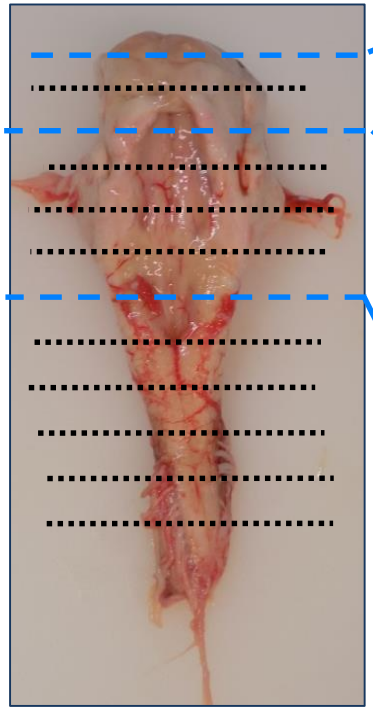
BRAINSTEM and CEREBELLUM FRESH SLICING

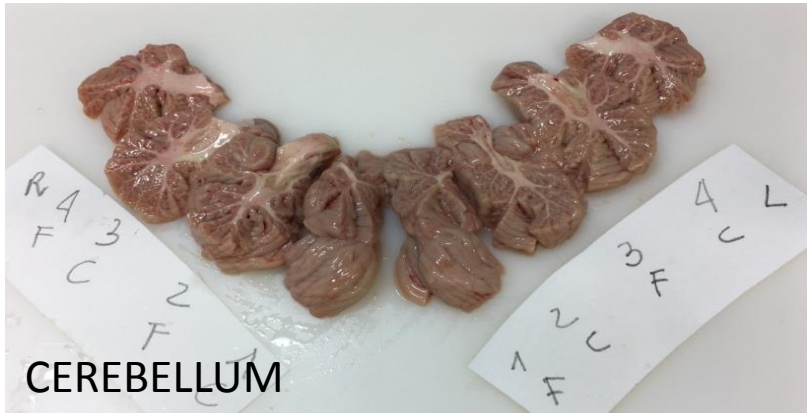
Banca del Cervello
PROGRAMMA DI RACCOLTA E STUDIO

anterior

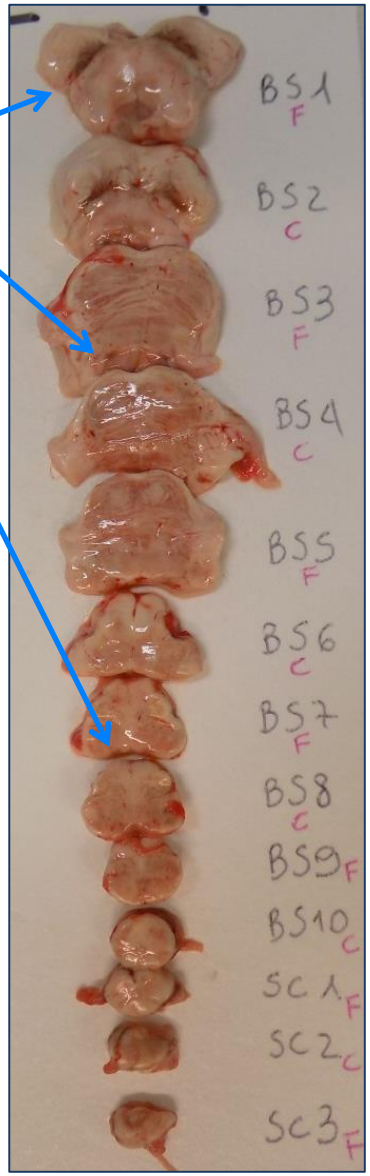


posterior





Left **Right**



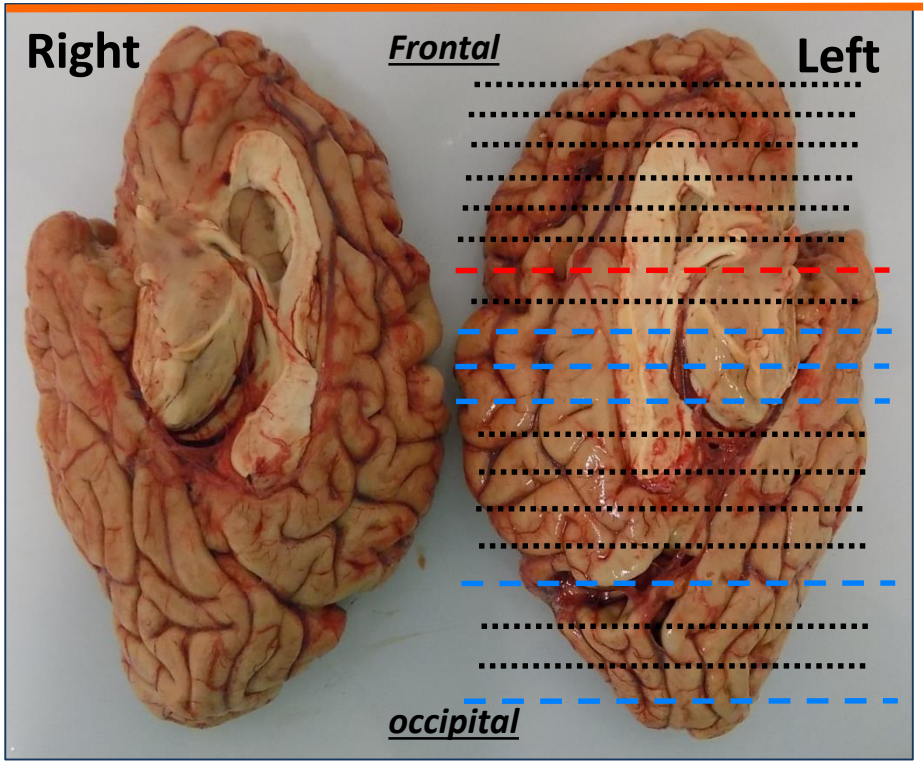
- **1° cut** through meso-diencephalic passage: ***SUBSTANTIA NIGRA***
- **2° cut** rostral pons, close to superior apex of IV ventricle: ***LOCUS COERULEUS***
- **3° cut** over the inferior apex of IV ventricle: ***DORSAL MOTOR NUCLEUS OF VAGUS***

8 mm slices (about 10 for brainstem and 3 for spinal cord), fixed or frozen alternately

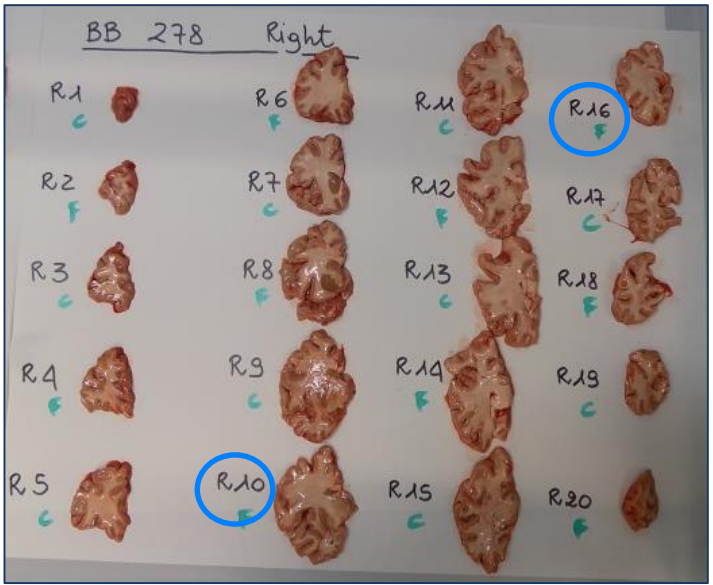
BRAIN FRESH SLICING

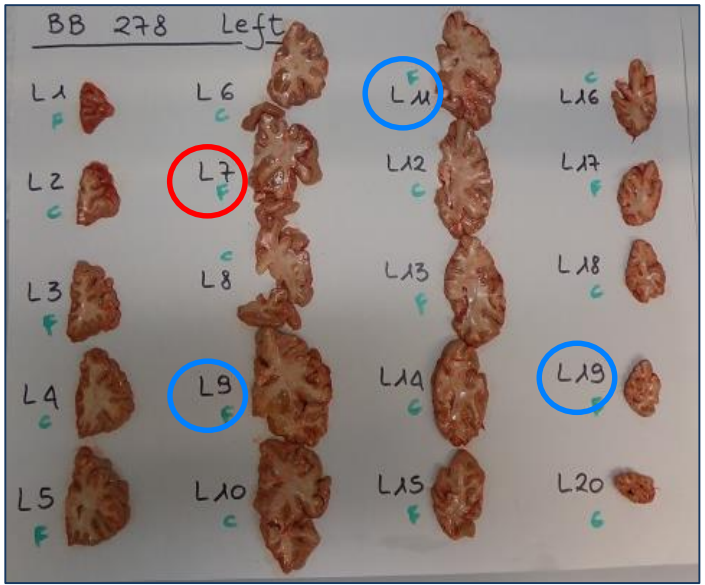
Banca del Cervello
PROGRAMMA DI RACCOLTA E STUDIO

8 mm slices (about 20 per hemisphere): alternate sections from each hemisphere are retained as fixed or frozen material



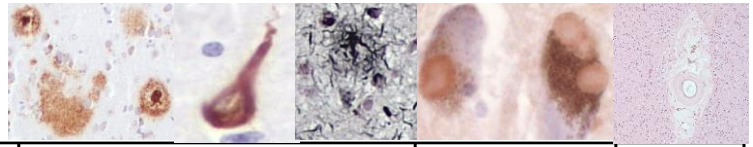
- **Central Cut** (just behind optical chiasm): **FRONTAL, CINGULATE, BASAL GANGLIA, MAYNERT NUCLEUS, AMYGDALA**
- **Charcot Cut** (through mammillary bodies): **BASAL GANGLIA, THALAMUS, ANT. HIPPOCAMPUS**





- **Temporal Cut** **TEMPORAL, POST. HIPPOCAMPUS, ENTORHINAL cortex**
- **Parietal Cut** **PARIETAL LOBULE**
- **Occipital Cut** **STRIATAL cortex**

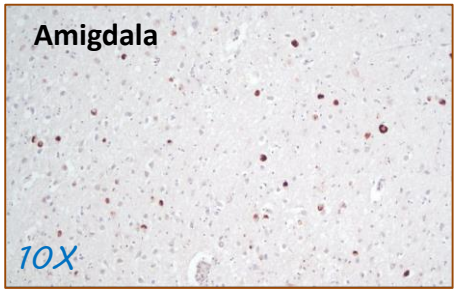
Banca del Cervello
PROGRAMMA DI RACCOLTA E STUDIO

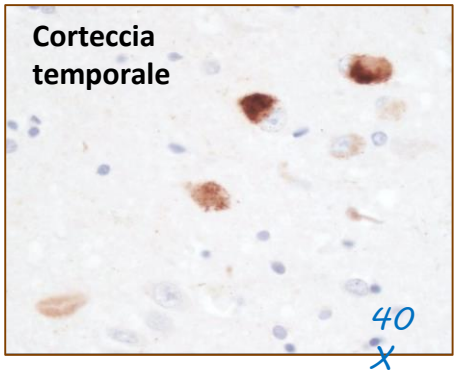


Montine '12	AD Neuropathologic Change			LBD	VBI & HS
	A	B	C		
Region	Stain for Aβ/amyloid plaques [57]	Stain for NFTs [14,15]	Stain for NPs [41]	Stain for LBs	H&E
Medulla including DMV				1°: IHC or H&E [3]	VBI
Pons including LC				1°: IHC or H&E [3]	VBI
Midbrain including SN	3°: if 2° is +			1°: IHC or H&E [3]	VBI
Cerebellar cortex and dentate n.	3°: if 2° is +				VBI
Thalamus and subthalamic n.[1]					MVL
Basal ganglia at level of AC with basal nucleus of Meynert[1]	2°: if 1° is +	Consider[4]			MVL
Hippocampus and EC[1]	2°: if 1° is +[2]	Yes	Consider[4]	2°: IHC in at least one if 1° +	HS
Cingulate, anterior					VBI
Amygdala				1°: IHC [3]	VBI
Middle frontal gyrus[1]	1°[2]	Yes	Yes	2°: IHC in at least one if 1° +	MVL
Superior & middle temporal gyri[1]	1°[2]	Yes	Yes		MVL
Inferior parietal lobule[1]	1°[2]	Yes	Yes		MVL
Occipital cortex (BA 17 & 18)[1]	Consider[4]	Yes	Consider[4]		MVL
WM at ACA, MCA, and PCA watershed					Consider[4]

We perform **pTDP-43** IHC on the following sections: amygdala, hippocampus, entorhinal and temporal cortex. In cases of possible FTLD we examine also the frontal lobe

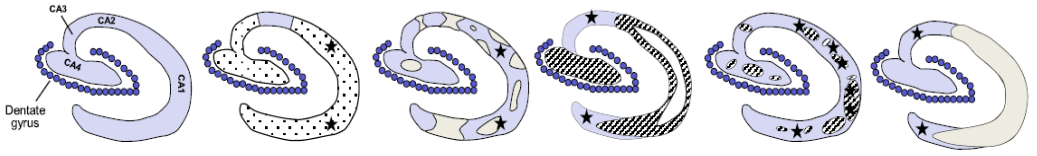
Alafuzoff '15





TDP-43 (TAR DNA-binding protein of **43** kDa)

In neurodegenerative disorders (FTD-ALS-AD) TDP43 undergoes phosphorilation and ubiquitination leading to inclusions bodies formation, mainly in the cytoplasm and neurites (NCIs, DNs)

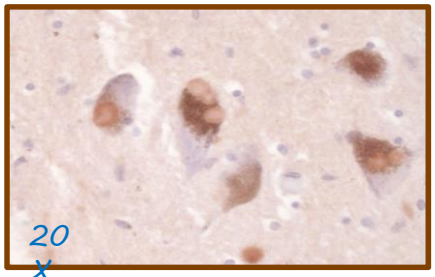


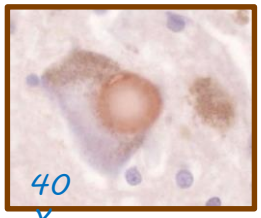
Rauramaa '13

p62 immunohistochemistry (or hyperphosphorylated tau, TDP43, α-synuclein) should be applied to screen for ★

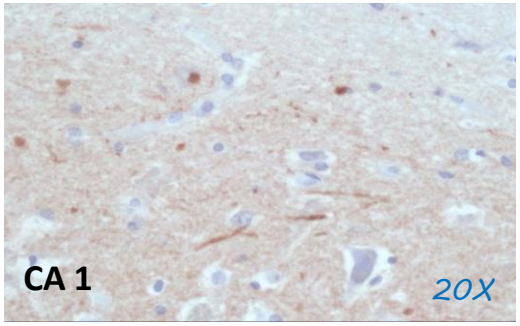
Hippocampal lesions: **1 and 2** correspond to microinfarcts and infarcts; **3** e **4** represents hippocampal sclerosis **HS** (moderate to severe neuronal rarefaction and atrophy). In case of HS, we point out the presence or the absence of degenerative lesions such as pTAU, TDP-43, alfa-syn.

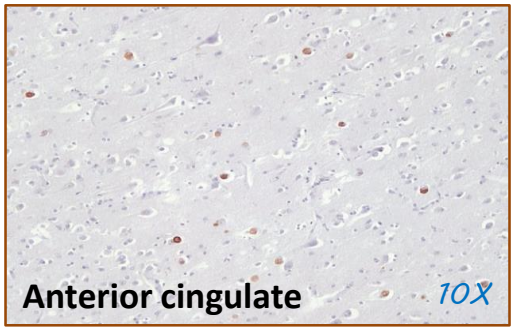
Lewy Type Synucleinopathy (LTS): IHC α-syn





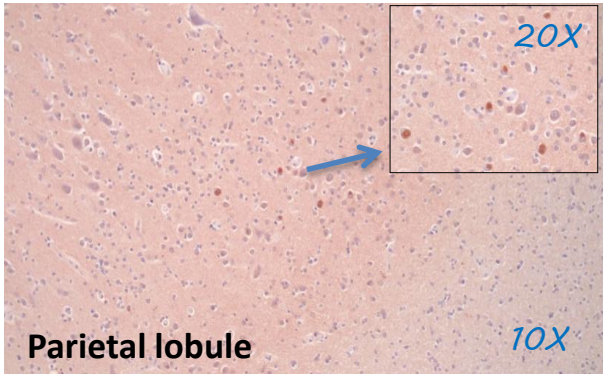
Brainstem (SN)



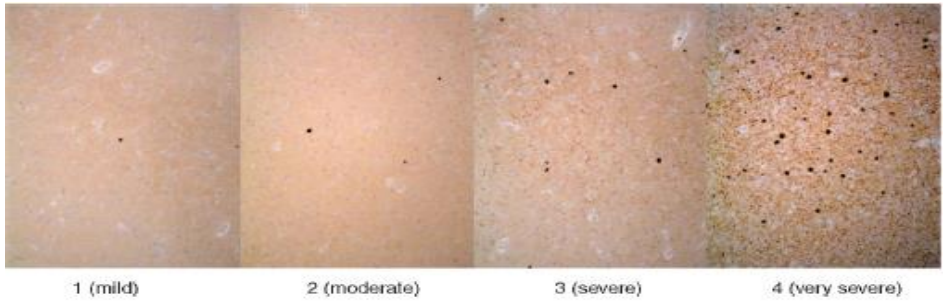


Limbic

Neocortical



Regional Score (DLB III)	I. Olfactory Bulb-Only	IIa. Brainstem Predominant	IIb. Limbic Predominant	III. Brainstem/Limbic	IV. Neocortical
Olfactory Bulb	Score 1–4	Score 0–4	Score 0–4	Score 0–4	Score 0–4
Brainstem	Scores all 0	Either a or b a. Scores 1–2 b. Scores 3–4	Either a or b a. Scores 0 b. Scores 1–2	Either a or b a. Scores 1–2 b. Scores 3–4	Scores 0–4
Limbic	Scores all 0	Match a & b with above a. Scores 0 b. Scores 1–2	Match a & b with above a. Scores 1–2 b. Scores 3–4	Match a & b with above a. Scores 1–2 b. Scores 3–4	Scores 0–4
Neocortical	Scores all 0	Scores 0–1	Scores 0–1	Scores 0–1	Scores 2–4



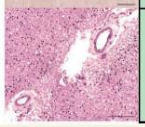
Clinical categories: ILBD (I, IIa), PD (IIa), ADLBD (more frequently IIb), PDD (more frequently III), LBD (IV)

Beach ’09 ; McKeith ’05 ; McKeith ’17

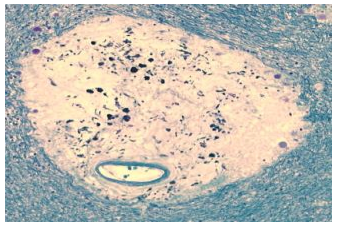
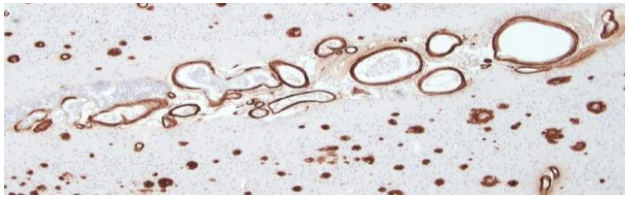
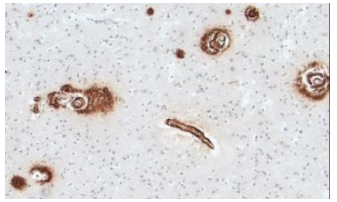
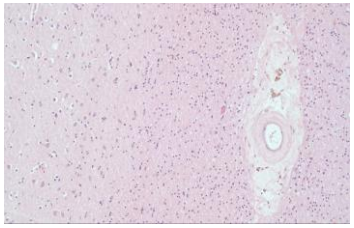
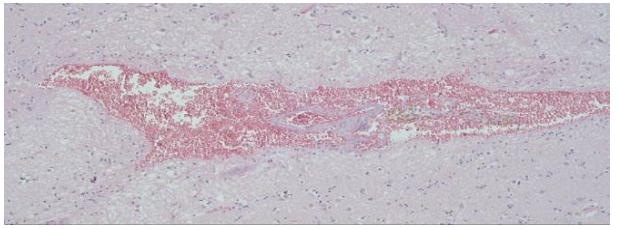
Hierarchy for microscopic evaluation of LTS:

1) olfactory bulb, brain stem (SN, LC, DNV), amygdala, and temporal lobe;
2) hippocampal formation, entorhinal cortex, anterior cingulate, middle frontal, and inferior parietal lobule.
If clinical features of cortical LBD are present (i.e. fluctuations and/or hallucinations), we consider limbic structures and isocortex for the first step.

Vascular pathology: microscopic features

Likelihood that cerebral vascular disease contributed to cognitive impairment		Low (<50%)			Moderate (50-80%)		High (>80%)		
One or more large (> 10 mm) subcortical cerebral infarcts		-	-	-	+	-	+	+	+
Moderate or severe occipital leptomeningeal CAA		-	+	-	-	+	+	-	+
Moderate or severe occipital white matter arteriolosclerosis		-	-	+	-	+	-	+	+

Score	Staging
Frontal and temporal lobes	
0	Normal appearance of brain, vessels, white matter, and cortex
I	Mild modification of vessel walls, perivascular spaces, or white matter
II	Moderate to severe but isolated modification of the vessel walls (arteriolosclerosis or amyloid angiopathy), usually associated with hemosiderin deposits in the perivascular spaces
III	Moderate to severe perivascular space dilatations either in the deep or the juxtacortical white matter
IV	Moderate to severe myelin loss
V	Presence of cortical microinfarcts
VI	Presence of large infarcts
Hippocampus	
0	Normal appearance
I	Mild modification of vessel walls or perivascular spaces
II	Moderate to severe perivascular space dilatations
III	Presence of microinfarcts (usually in Ammon horn or the subiculum)
IV	Presence of large infarcts
Basal ganglia	
0	Normal appearance
I	Mild modification of vessel walls or perivascular spaces
II	Moderate to severe perivascular space dilatations
III	Presence of microinfarcts
IV	Presence of large infarcts
Total vascular score	
Frontal lobe + Temporal lobe + Hippocampus + Basal ganglia (/20)	

We examine at least two hemispheric macro-sections: 1) Charcot's cut (frontal and temporal lobes, basal ganglia, anterior thalamus); 2) cut through the occipital lobe. Furthermore we consider the hippocampal formation, one cerebellar section, and all three principal sections of the brainstem. For CAA we use Lowe's scoring (0-3 +/- capCAA)

Stains used: H&E, Luxol, Immunohistochemitry: GFAP, 4G8

Skrobot- Attems 2016, Lowe 2014 , Deramecourt 2012

doi:10.1093/brain/awv191 BRAIN 2015: 138; 2732–2749 | 2732
BRAIN
A JOURNAL OF NEUROLOGY

The behavioural/dysexecutive variant of Alzheimer's disease: clinical, neuroimaging and pathological features

Rik Ossenkoppele,[1,2,3,4] Yolande A. L. Pijnenburg,[3] David C. Perry,[1]

doi:10.1093/brain/aww005 BRAIN 2016: 139; 1211–1225 | 1211
BRAIN
A JOURNAL OF NEUROLOGY

MRI visual rating scales in the diagnosis of dementia: evaluation in 184 post-mortem confirmed cases

Lorna Harper,[1] Giorgio G. Fumagalli,[2] Frederik Barkhof,[3] Philip Scheltens,[4]

NEUROPATHOLOGY
Neuropathology 2017; **37**, 150–173 doi:10.1111/neup.12364

Symposium: Fundamentals learned from diversity among typical and atypical appearances

Typical and atypical appearance of early-onset Alzheimer's disease: A clinical, neuroimaging and neuropathological study

Shinobu Kawakatsu,[1,2,3] Ryota Kobayashi[2,3] and Hiroshi Hayashi[2,3]

Neurobiology of Aging 63 (2018) 75–87
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Neocortical origin and progression of gray matter atrophy in nonamnestic Alzheimer's disease

Jeffrey S. Phillips[a,b,*,1], Fulvio Da Re[a,c,d,1], Laynie Dratch[a], Sharon X. Xie[e],

"… post-mortem confirmed cases as the gold standard …"

"… most cases of PCA which were investigated postmortem revealed the histopathological lesion type of Alzheimer's disease …"

"… nonamnestic Alzheimer's patients have relative sparing of the hippocampus, but the pattern in which the disease spreads is unclear. We selected 129 patients with pathology confirmed by autopsy … "

LA RICERCA HA BISOGNO DI DIAGNOSI DEFINITE

Le sindromi cliniche sono definite dalle sede delle lesioni più che dalla loro natura molecolare. Definire la natura delle lesioni e, quindi, la loro patofisiologia richiede il riscontro istopatologico (conferma dell'efficacia diagnostica dei biomarcatori)

Banca del Cervello
PROGRAMMA DI RACCOLTA E STUDIO

Attualmente 22 cervelli: 20 esaminati e 2 *in progress*

1	BB37	Major Neurocognitive Disorder due to Alzheimer disease	High AD pathology, moderate SVD, TDP43+, ctx LTS, HS - MIXED DEMENTIA
2	BB105	Major Neurocognitive Disorder due to Alzheimer disease	High AD pathology - AD
3	BB137	Major Neurocognitive Disorder due to multiple etiologies (Alzheimer's Disease and Vascular disease)	High AD pathology, SVD , amyloid angiopathy (CAA) - MIXED DEMENTIA
4	BB181	Major Neurocognitive Disorder due to Alzheimer disease	Intermediate AD pathology, mildSVD - leptoCAA, Lewy's Bodies dementia (LBD - sev. LTS) - MIXED DEMENTIA
5	BB115	Major Neurocognitive Disorder due to Alzheimer disease	Intermediate AD, moderate SVD, Inflammation, incidental LB, Beach IIa, HS - MIXED DEMENTIA
6	BB23	Major Neurocognitive Disorder due to vascular disease	Intermediate AD pathology, Severe and widespread CAA - MIXED DEMENTIA
7	BB102	Major Neurocognitive Disorder due to vascular disease	Vascular Dementia, incidental LB - VAD
8	BB224	Major Neurocognitive Disorder due to multiple etiologies	Low AD, TAU pathology - PART, incidental LB in brainstem - NFT DEMENTIA
9	BB47	Major Neurocognitive Disorder due to multiple etiologies (Lewy Bodies Dementia and Vascular disease)	High AD pathology, widespread TAU pathology,- ARTAG - AD/CBD
10	BB153	Normal aging; death due to colon cancer with widespread metastatic diffusion	Low AD pathology, mild SVD - NO DEMENTIA
11	BB118	Normal aging; death due to cancer of liver (HCC)	Moderate SVD - NO DEMENTIA
12	BB236	Major Neurocognitive Disorder due to Alzheimer disease	High AD pathology, Severe basal ganglia SMV (several microbleeds) - MIXED DEMENTIA
13	BB138	Major Neurocognitive Disorder due to multiple etiologies (Alzheimer's Disease and Vascular disease)	High AD pathology, SVD, amyloid angiopathy (CAA), limbic TDP43 - MIXED DEMENTIA
14	BB109	Mild-NCD due to multiple etiologies, brain tumor	Low AD pathology, incidental LB pathology in SN, mild SVD - NO DEMENTIA
15	BB271	Major Neurocognitive Disorder due to Alzheimer disease	Intermediate AD, moderate BG SVD, sporadic MB and leptoCAA, limbic LTS, TDP43 (Josephs '14: I) - MIXED DEMENTIA
16	BB71	Normal aging; death due to heart failure	In progress
17	BB189	Major Neurocognitive Disorder due to Alzheimer disease	Intermediate AD, moderate BG SVD, CAA-capCAA, TDP43 (Josephs '14: V) - MIXED DEMENTIA
18	BB278	Major Neurocognitive Disorder probably due to Lewy Bodies Dementia	Intermediate AD, moderate neoctx LTS, moderate-severe TDP43, HS - MIXED DEMENTIA
19	BB247	Major Neurocognitive Disorder due to multiple etiologies	TAU pathology - PART - ARTAG, HS - NFT DEMENTIA
20	BB85	Major Neurocognitive Disorder probably due to Lewy Bodies Dementia	Intermediate AD, Moderate SVD, severe CAA-capCAA, severe limbic LTS, moderate HS - MIXED DEMENTIA
21	BB14	Major Neurocognitive Disorder due to multiple etiologies	Intermediate AD pathology, Moderate vascular pathology - MIXED DEMENTIA
22	BB282	Major Frontotemporal Neurocognitive Disorder	In progress

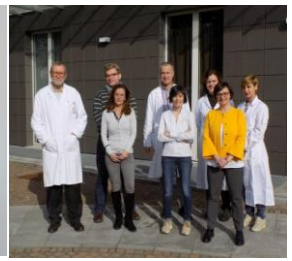
- **Genomica**: mediante GWA (Genome Wide Association) determinazione di mutazioni e SNPs (fattori di rischio genetico, medicina personalizzata)
- **Epigenomica**: modificazioni anche reversibili della struttura biochimica del DNA che non modificano la sequenza ma ne influenzano la trascrizione (metilazione, acetilazione, miRNA)
- **Trascrittomica**: analisi dell'RNA trascritto, indicatore dei geni attivi in una certa area e condizione fisiologica o patologica
- **Proteomica**: struttura, funzione e interazione delle proteine (topografia e diffusione del *misfolding* proteico, studi di vulnerabilità cellulare in aree specifiche)
- **Lipodomica**: composizione e struttura dei lipidi delle membrane neuronali (comparazione con lipidi circolanti e Apo-lipoproteine)
- **Metabolomica**: studio del profilo delle molecole elementari

INTERATTOMA … TECNICHE DI *DEEP LEARNING*

Conclusioni

- La diagnosi definita è sempre clinica e neuropatologica
- Studi longitudinali che includano la neuropatologia (diagnosi definita) sono necessari per la ricerca sulle malattie neurodegenerative: correlano i dati clinico-epidemiologici con quelli neuropatologici (istologici e omici)
- Trovare correlazioni tra dati di contesto ambientale, dati clinici, dati genetici, istologici e omici: nessi di causalità
- Costruire modelli prognostici e di intervento anche mediante tecniche di *machine learning*




Thanks to THE BRAIN BANK TEAM