REVIEW

Use of PIXYL software analysis of brain MRI (with & without contrast) as valuable metric in clinical trial tracking in study of multiple sclerosis (MS) and related neurodegenerative processes

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Abstract

Multiple sclerosis (MS) is a complex inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltrates lead to damage of myelin and axons. Often this seems associated with a type of autoimmune and inflammatory disorder which is transient, with attempted remyelination that is not durable. Neurological disorders appear somewhat randomly, seem to improve and demonstrate recovery. Progression is variable, but over time, seems to become more widespread with microglial activation associated with extensive chronic neurodegeneration and persistent disability. Magnetic resonance imaging (MRI), with and without contrast, has become the standard metric of finding and following lesions and axonal loss. The traditional methods of treatment include medications, diet, exercises, and neurological supportive care. The medications are limitedly effective, and often have very undesirable side effects. Medications seem to reduce the frequency of new episodes, but are not yet able to reverse acquired deficits or long-term progression. Clinical trials exploring cellular therapy are currently underway, and showing some interesting progress with management of many with the MS disorder. What has been needed is a reliable and affordable metric to track the patient's clinical progress, both from a location of lesion and volumetric changes on MRI. PIXYL software offers a major improvement in providing tracking capability of outcomes in use of medications or cellular therapy. Time consuming, limited accuracy, and costs of having serial MRI segmented and analyzed make standing neuroradiological interpretations difficult to standardize. This software offers a new ability to resolve much of these issues, including taking manual (human) interpretation out of potential bias or different interpreters, and offers volumetric and lesional changes over time in serial studies. This paper introduces the functions and values of use of sophisticated software analytics in the evaluation and management of this ongoing disease process.

Key words: MS; brain MRI; degenerative neurological disorders

OVERVIEW OF MS

Multiple sclerosis (MS) is an inflammatory, demyelinating condition of the central nervous system (CNS) which is believed associated with autoimmune changes.[1-3] Specifically, white matter tracts are affected, including those of the cerebral hemispheres, infra-tentorium, and spinal cord areas. They may occur in multiple areas, therefore, the clinical presentations are often diverse. These lesions, called "plaques" form in CNS white matter, often present with physical disabilities and cognitive decline.[4,5]

Magnetic resonance imaging (MRI) has revolutionized the diagnosis and monitoring of MS patients. Its sensitiv-

ity and interpretations are help by use of contrast media, evaluations in patients having had a single episode of neurologic impairment who do not meet the clinical criteria for diagnosis.[6] Use of 3-dimensional (3-D), T2* weighted, gradient-echo ((T2*GRE) and white matter-attenuated, turbo-field-echo (TFE) sequences at 7T field strength is very effective at recording most cortical lesions.[7] MRI contrast use helps identify variations reflecting alterations in brain iron and myelin.[8,9]

Inflammation and breakdown of blood-brain barrier occurs in MS lesions, leading to changes in extravascular fluid produces hyper-intensity of the T2-weighted images and reported as hyper-intense white matter lesions. T1-weighted images are seen as dark (hypo-intense) images caused by tissue with more water, while high fat content (white matter) and appear bright.

Newer MRI pulse sequences and techniques, such as fluid-attenuated inversion recovery (FLAIR), uses a heavily T2-weighted technique which dampens the ventricular cerebrospinal fluid (CSF) (*i.e.*, free-water). This leaves brain parenchymal lesions (*e.g.*, plaques of MS), leaving the CSF appearance as black.

The PIXYL software algorithms are capable of interpretations using these modalities, with accuracy that rivals, or may exceed, manual readings. When comparing a series of MRI in the same patient, this is a huge time-saver and cost effective means of tracking. For those clinical trial investigators who are neither expert in reading, nor radiologically backgrounded, this is a major contribution.

OVERVIEW OF IRB NCT#02939859

The purpose of the trial design is to determine safety and efficacy of use of autologous, adult, adipose-derived cellular stromal vascular fraction (AD-cSVF) suspended in normal saline (NS) and delivered intravascularly in cases of documented multiple sclerosis (MS) (**Figure 1**). Since the capabilities of the AD-cSVF have been shown to possess immune modulation and inflammatory modulation, it seemed to offer some possibilities which warrant clinical
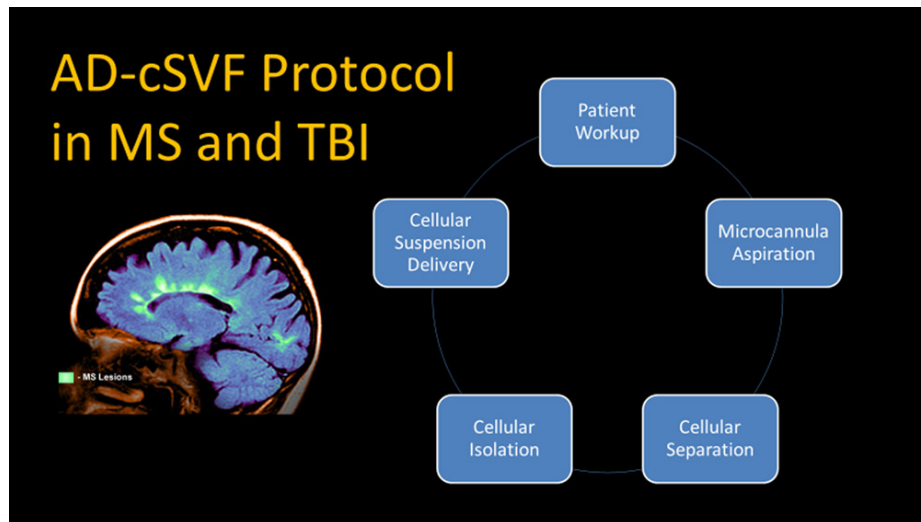

Figure 1: Clinical trial outline of treatment.
Note: AD-cSVF: Adipose-derived cellular stromal vascular fraction; TBI: traumatic brain injury.
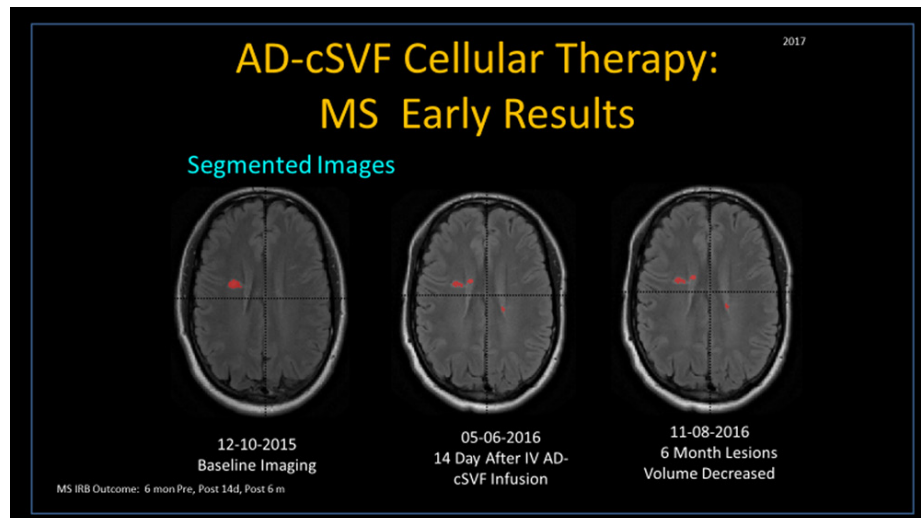

Figure 2: Multiple sclerosis (MS) trial patient at: Right – six month pre-cellular therapy; Middle – two weeks post-cellular therapy; Left – six months post-cellular therapy.
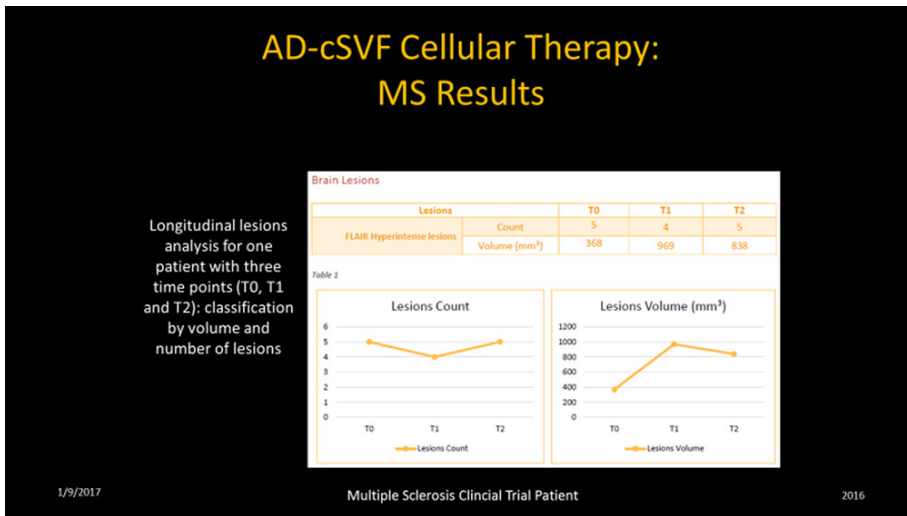Note: AD-cSVF: Adipose-derived cellular stromal vascular fraction.

Figure 3: Lesion count in multiple sclerosis (MS) trial patient at same intervals.
Note: The counts initially decline, then returned and volumes went down at six month post-cellular therapy. AD-cSVF: Adipose-derived cellular stromal vascular fraction.
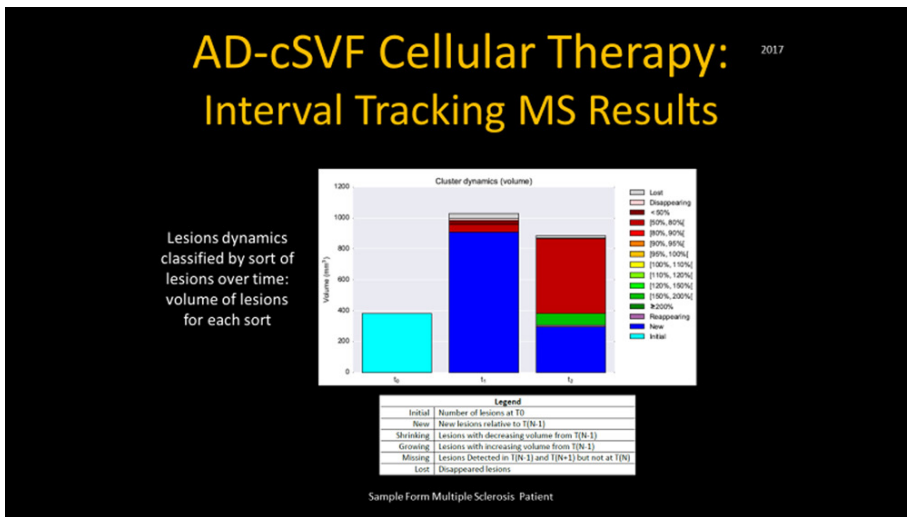

Figure 4: Sample of multiple sclerosis (MS) patient showing lesion dynamics at intervals tested. (See below image cluster dynamic of lesion changes showing).
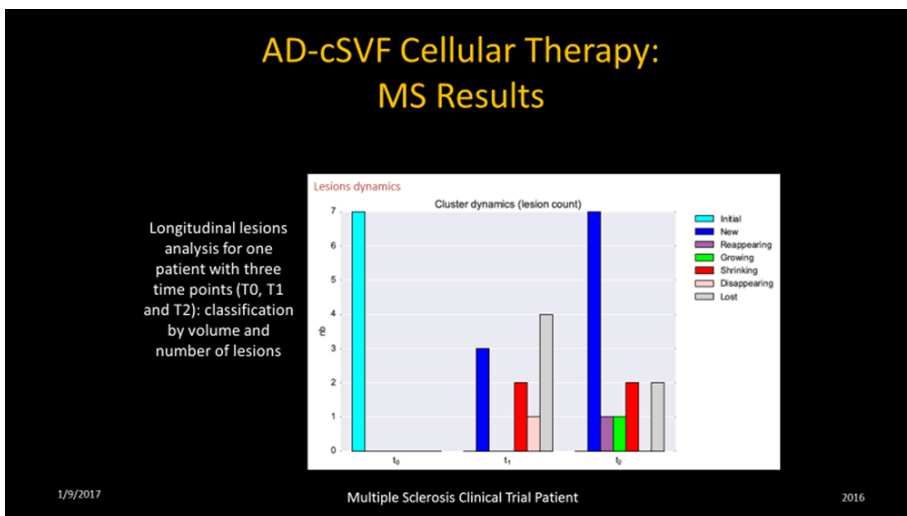Note: AD-cSVF: Adipose-derived cellular stromal vascular fraction.

Figure 5: Sample of multiple sclerosis (MS) trial patient reporting cluster dynamics (counts and activity) initial, new, reappearing, growing, shrinking, disappearing, and lost categories.
Note: AD-cSVF: Adipose-derived cellular stromal vascular fraction.

trial examination.[10,11]

In design of the protocol, evaluation of relative points were determined to include magnetic resonance imaging (MRI with/without contrast). This is an accepted metric in common use amongst neuro-radiologists and neurologists in diagnostic and monitoring applications in management of progressive MS patients.[12] There is a general reluctance for some neurological specialists to order repetitive MRI due to the limitation of therapeutic alternatives other than diet, physical therapy, and medications. Unfortunately, many of the available medications have very undesirable side effects, and often prove of limited efficacy. When dealing with patient follow up examinations, several specialist that were contacted said the studies were expensive and there was nothing particular they could do with the patients based on the MRI changes.

In order to evaluate the potential changes that could be attained, it was decided that we needed a method of documenting changes in sizes, locations, or volumes of the lesion (plaques) found. That led to discussions with a European company (PIXYL) who developed a software program specifically capable of repetitive analysis, which provided the study a high quality interval correlative studies and the ability to thereby "track" lesions without having to have multiple interval segmenting by radiologists in each case. No only is this very time consuming and subject to human error, it is prohibitively expensive to be practical. The software and its complex mathematical iterations, prove to be consistent, accurate, and considerably less difficult to acquire analyses.

These analyses permit location, number, sizing, status of lesions (new, existing, growing, declining, fragmenting (lower volume over previous exam), and lost lesions in comparative fashion). It is considered imperative to distinguish a variety of lesions (size, location, texture of each). This is not a simple interpretation due to the variety of aging, vascular changes, locations, *etc*.. In development of the software protocols, the assumption was that manual segmentation was the gold standard, and the goal of the PIXYL analysis was to compare to the "ground truth" (true abnormal voxels) to the similarity index and evaluating the ROC curves. The differences were minimized and the outcome analytics showed an excellent correlation.

The comparison between brain-damaged versus healthy subjects was determined that differences only related to presence or absence of abnormal tissue. These then were used to attempt to cancel or minimize sources of differences by demographic variables (age, gender, *etc*.) and to insure an accurate segmentation or normalization protocols.[13-15]

Since most clinical trials in this subject may not include expert radiologists on staff, or not be able to reasonably afford repetitive analytics on interval images, the alternative and standardization offered by PIXYL utilization.

EXAMPLE OF MS CLINICAL TRIAL TRACKING USING PIXYL SOFTWARE ANALYSES

As a simplification of a clinical trial in MS, the hypothesis is to evaluate the ability of use of adipose-derived cellular stromal vascular fraction (AD-cSVF) devoid of the adipose stroma itself (cell separated). Simple closed-syringe microcannula lipoaspiration from the subdermal deposit of abdomen and flanks (male & female) or upper thighs (females) is utilized to gently extract 50–100 cc of tissue stromal vascular fraction (AD-tSVF). This product is centrifuged to compress the adipose layer from the extraneous carrier fluid and free lipid gradient. Following removal of the debris and carrier (tumescent) fluid excess, the sample is mixed in a 1:1 ratio of GMP/GLP, sterile, blend of collagenases-neutral protease reconstituted enzyme. This is then incubated and agitated to encourage gentle cellular separation, followed by centrifugation and palletization of the heterogeneous, autologous cellular components. Final step includes thorough rinsing and binding of the cell pellet to effectively remove residual collagenase from the pelletized stromal cells (without matrix). This pellet is then suspended in sterile Normal Saline (NS), and slowly returned to the patient *via* peripheral intravascular route over 30–45 minutes period. The product introduced is tested for viabilities and cellular number prior to delivery, and recorded for a part of the trial data.

The logic of this protocol is to test for safety and efficacy of autologous cellular infusions as a potential treatment aid, or in compliment with traditional medications. The AD-cSVF cellular complex has been shown to be potentially important is immune modulation and inflammatory changes which are considered of value with this group, as well as inflammatory or autoimmune-related diseases. This particular trial is anticipated to report within 5 years, following tracking and compiling of metrics of outcomes. It is our belief that the PIXYL software contribution with the MRI tracking component may prove to be of very high value.

SAMPLE MRI UTILIZATION IN MS

Example of 32 year old progressive MS patient intolerant to any medications (severe reactions, hospitalized). On diet and standard physical therapy protocols by neurologist. Images taken at 6 month prior to treatment, with patient developing worsened symptoms. Two weeks after clinical trial protocol, and at 6 months MRI (w/wo contrast) are demonstrated (**Figures 2–5**). In addition, the lesion and volumetrics demonstrated a reduction in volume of the lesions, with apparent fragmentation of the plaques. Another sample will be run at 1 year to determine interval changes. The patient clinically was markedly improved in all areas of clinical responses.

PIXYL Software (Tracking in MRI Clinical Trial)

PIXYL software designed for medical research and practice is designed to consistently extract the maximum information contained in MRI brain scans. Its complex solution specifically is able to locate, identify, and quantify a wide range of lesion changes related to pathology (MS, stroke, degenerative disorders and traumatic brain injuries (TBI).[16] The tools developed are able to automatically identify and measure healthy brain tissue and lesions, based on densities, without the variability introduced by manual segmentation analysis. Such manual delineations are difficult to reproduce, time consuming and very costly needs. The software is unique, with its abilities to located region of the lesions, then classify into "sub-regions" to be able to calculate volumes, using statistical modeling algorithms based on advances in neuroimaging, classification and distributive computer.[17] With proven abilities to accurately track and measure lesion volumetrics in the brain, without the common interpretation variances, the attraction to apply this technology to tracking in clinical trials and practice is obvious. Consistency is very important in these interpretations, making the proven software modeling in patients a great advance.

Discussion

Due to frequency and many difficulties associated with MS varients, improvement in our tracking of patient progress, finding a more standardized analytic for lesion identification and progress is considered of immense potential and value.

As there is no single test or exam that is fully diagnostic for MS, or which has the ability to determine extent or lesional progress of the changes found in MS. The traditional principles of MS diagnosis are typically based on revealing of asymptomatic dissemination of white matter lesions in space and over time. The MRI is generally considered the most sensitive method of revealing the pattern of dissemination in space (DIS) and time in space (DIT) and making early diagnosis of MS possible.[18] This is accomplished with a good sensitivity rate, approaching 94% if within the first year, with a specificity of 83%. PIXYL software also plays an important role in diagnostics to rule out alternative issues such as spinal stenosis, stroke, TBI, or tumors.

The characteristic lesions of MS on MRI are Dawson Fingers, ovoid lesions, corpus callosum lesions some asymptomatic spinal cord lesions. Combination of correlation of sequential images with an automated analysis, clinical changes, and evaluation of volumes of these lesions is felt to provide a major contribution to MS patient management in the future. For many years, many neurologists have elected to rarely retake MRI (with/without contrast) due to not really having an alternative treatment based on the studies. Only when exacerbations or major changes became manifest, they do try to re-establish a new baseline, but only for potential changes in traditional medications and therapies.

Use of contrast media, such as Gadolinium, is often helpful in evaluations of new lesions and activity, mass effects of lesions, and ability to enhance and evaluate meningeal changes. It provides useful information about new lesions, and helps rule out neoplasm, vascular malformations or leptomeningeal disease. It is likewise, of evaluation of the DIS and DIT changes. Volumetric evaluations are important, particularly in the face of lesion growing or receding.

Conclusion

Use of the PIXYL software analytics may prove of great value, both in the clinical and research settings. Neurologist who often depend on a variety of neuro-radiologists or radiologist to read studies. The variability of findings in manual segmentation with a single interpreter alone is significant. The ability to provide a serial analytic protocol, makes the confidence factor grow, knowing that a portion of the variability can be managed.

In doing interval testing, such as 6-month or 1-year examples and the ability to have a comparative volume, lesion analysis, and monitoring changes is of great value. In the clinical trial setting, the efficacy of utilization of either medications or cell based therapeutics can be examined to track the progress or decline in each patient, and provide us the ability to gather enough data to prove the efficacy of any given modality.

Much is left to be done in longer-term tracking of outcomes, but the cell-based protocols being studied will benefit from this objective analysis. Early changes are encouraging, but many more examples are needed to be conclusive or guide the many patients with the disorder to determine if this is a viable alternative to traditional approaches we are now utilizing.

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