Journal of Molecular Imaging & Dynamics
ISSN: 2155-9937

Prasad et al., J Mol Imag Dynamic 2012, 2:1
DOI: 10.4172/2155-9937.1000104

Research Article Open Access

Preliminary Evaluation of Potential Disease Modification by Hylan G-F 20 (Synvisc®) Using dGEMRIC

Pottumarthi V. Prasad[1]*, Wei Li[1], Thomas Schnitzer[2], Nitya Krishnan[3] and Deborah Burstein[3]

[1]*NorthShore University HealthSystem, Evanston, IL, USA*
[2]*Northwestern University's Feinberg School of Medicine, Chicago, IL*
[3]*Beth Israel Deaconess Medical Center, Boston, MA*

Abstract

The objective of the present study was to utilize delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC), as a reflection of cartilage matrix integrity, to evaluate the effects of a specific hyaluronan, hylan G-F 20 (SYNVISC®; Genzyme Biosurgery, Cambridge, MA) in OA patients over the course of 6 months, and to utilize these data to determine effect size and power calculations for future studies. An open-label, single-blind exploratory study of patients having knee OA (Trail registration: NCT00949494) was performed in thirty subjects (20 active, intra-articular injections (3, week apart) with hylan G-F 20; 10 control). dGEMRIC data was obtained at baseline, 3 and 6 months. No changes were measurable with hylan administration under the clinical and imaging conditions utilized in the current study. The lack of response in the current study may be because the subjects had a relatively advanced stage of disease with respect to cartilage integrity.

Keywords: Cartilage; Knee; Hyaluronans; Hylan; MRI; dGEMRIC

Introduction

The prevalence of osteoarthritis (OA) is increasing, and it is estimated by 2030 there will about 67 million Americans affected by this disease resulting in a substantial financial burden to society [1]. Although surgical intervention relieves the pain associated with OA and improves functionality, it is not a viable option for many. Non-surgical options include life style modifications, physical therapy, systemic anti-inflammatory medicines, intra-articular injections of steroids, and hyaluronic acid (HA) viscosupplementation. While all these are in general considered to be directed toward symptom modification, there is some speculation as to the potential disease modification afforded by viscosupplementation [2,3]. Additionally, there has been research driven towards developing disease modifying osteoarthritic drugs (dMOADs) mostly targeted towards cartilage [4].

One particular challenge in evaluating potential therapeutic disease-modifying agents has been defining structure-related endpoints for studies that are both adequately sensitive and specific. Because cartilage is central to the disease process, many techniques have focused on evaluating cartilage properties as an important determinant of OA and changes in these properties over time as a determinant of progression. Radiographic analysis of joint space width (JSW) is the currently accepted marker for evaluating articular cartilage thickness, and changes in JSW over time have been defined for control and OA populations and used as endpoints in trials of potential therapeutic agents [5]. MR imaging, which provides a measure of cartilage volume, has been proposed as a viable alternative to radiography [6], but not found to provide additional sensitivity to simple radiographic analysis [7]. Neither of these approaches directly addresses changes that are known to occur in cartilage matrix and that are central to the OA process, namely loss of integrity of collagen fibers and the decrease in proteoglycan concentration [8]. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is a technique that has been shown to be sensitive to such changes.

dGEMRIC is a method designed to image the distribution of fixed charge density in cartilage. It is based on the theory that if the negatively charged MRI contrast Gd-DTPA^{2-} (Magnevist, Bayer Healthcare, NJ, USA) is allowed to penetrate into cartilage, it will distribute in inverse relation to the concentration of the endogenous negative fixed charge, which is predominantly determined by the concentration of the charged glycosoaminoglycan (GAG) molecules. The GAG distribution would then be inversely related to the Gd-DTPA^{2-} concentration, or directly related to the T_1 values after penetration of the contrast agent into the cartilage, T_1(Gd) [9]. Studies *in vitro* have validated that proteoglycan estimates based on dGEMRIC measurements to be in very good agreement with biochemical assays or histology [10-16], and *in vivo* data that are consistent with the premise has been reported in animal models [17-20] and clinically [11,21].

Hyaluronans are therapeutic agents approved for the treatment of the pain associated with osteoarthritis [22]. In addition to providing joint lubrication and shock absorbancy as a constituent of synovial fluid, hyaluronan within cartilage serves as the backbone for the proteoglycans of the extracellular matrix. In the arthritic joint, the concentration and molecular weight of hyaluronans are decreased by 33% to 50%, limiting its role in maintaining normal joint biomechanics [22]. The purpose of viscosupplementation is to replace the lost hyaluronan and potentially stimulate the production of endogenous hyaluronan within the joint [23], although at the present time the exact mechanism of action is not completely understood [24,25].

Because of many studies documenting the effects of hyaluronans on cartilage metabolism, recent clinical investigations have focused on their ability to affect cartilage structure [3]. Most of these clinical trials have been limited by small sample sizes and lack of rigorous methodologies [3,4]; the one large clinical trial yielded inconclusive

***Corresponding author:** Pottumarthi V. Prasad, Ph.D, NorthShore University HealthSystem, 2650 Ridge Avenue, Walgreen Jr. Bldg., Suite G-507, Evanston, IL 60201, USA, Tel: 847-570-1349; E-mail: pprasad@northshore.org

Received January 09, 2012; **Accepted** February 03, 2012; **Published** February 06, 2012

Citation: Prasad PV, Li W, Schnitzer T, Krishnan N, Burstein D (2012) Preliminary Evaluation of Potential Disease Modification by Hylan G-F 20 (Synvisc®) Using dGEMRIC. J Mol Imag Dynamic 2:104. doi:10.4172/2155-9937.1000104

results [26]. The current pilot study was undertaken in an attempt to utilize a newer technology, dGEMRIC, shown *in vivo* to reflect cartilage matrix integrity, to evaluate the effects of one specific hyaluronan, hylan G-F 20 (SYNVISC®; Genzyme Biosurgery, Cambridge, MA), injected intra-articularly, compared to usual medical management on cartilage in OA patients over the course of 6 months, and to utilize these data to determine effect size and power calculations for future studies

Methods

Subjects

The study was approved by the institutional review board and all subjects gave informed consent prior to their participation. This was an open-label, single-blind (dGEMRIC data were analyzed blinded to study group allocation), exploratory study of patients having knee OA with stable, moderate levels of pain appropriate for management by intra-articular injection with hylan G-F 20. Patients meeting inclusion/exclusion criteria were randomly assigned to either active (intra-articular injection with hylan G-F 20) or control (continued usual care consisting of maintenance of existing oral and/or physical therapy) groups in a 2:1 ratio. Patients treated with hylan G-F 20 received the standard clinical dosage (3 injections, 1 week apart). Patients entered into the active arm of this trial underwent knee MRI (with dGEMRIC) prior to initiation of hylan G-F 20 therapy and then at 3 months and 6 months post-treatment. Controls had knee MRI examinations with dGEMRIC performed at baseline, 3 and 6 months but did not have knee arthrocentesis performed. Thirty subjects (20 active, 10 control) were enrolled; one in the active group did not complete the trial. Most knees included had a KL score of 2 or 3, considered to be mild and moderate OA respectively in current clinical practice.

MR Scanner and sequences

The study was performed on a 32-channel 1.5 T MR system (Magnetom Avanto, Siemens, Erlangen, Germany) using a commercial transmit/receive extremity knee coil. 2D IR-TSE sequence (TR/TE=2200/13ms, TI=1680, 650, 350, 150, 50 ms, matrix size=384x384, slice thickness=3mm, FOV = 16cm) was used to acquire data in one central slice in the medial and lateral condyles.

Clinical status

Patient Global Assessment was a pre-specified secondary endpoint that was collected at baseline and each subsequent visit using a 10-point Likert scale. A 10mm VAS pain scale was also administered at the screening, baseline, 3 month and 6 month visits to determine subject qualification in the study as well as assess response to treatment.

Data analysis

For each subject, T1(Gd) values were reported from full thickness regions of interest (ROIs) defined in the central femoral and tibial regions of the medial and lateral condyles (cMF, MT, cLF, LT) similar to a previous report [27]. T_1 mapping was performed offline using MRI Mapper based on MATLAB (Mathworks, Natick, MA). Similar analysis was also performed with just the superficial layer.

Statistical analysis

The primary end point was the change in dGEMRIC index, T1(Gd) over time and between groups. All descriptive statistics are presented as mean ± SD (standard deviation). The between-group (active *vs.* control) differences were assessed using a two-sample *t* test or a Wilcoxon two sample test, at each time point (month 0, 3 or 6), with respect to cLF, MT, cMF, and LT. Wilcoxon test was used if the normality assumption by Shapiro-Wilk test was not satisfied. The change over time was assessed by constructing a mixed-effect model (or repeated ANOVA) which included group, time (month 0, 3 and 6) and interaction of group by time as fixed effects and individual subject as random effects. SAS 9.2 (Cary, NC) was used to perform all the analyses and a p value < 0.05 was regarded as statistically significant.

Effect size (ES) of the change over time for each of the compartments was evaluated by computing the variance of the means divided by the square of the within-group (control) error term. An effect size 0.01~0.06 was considered as small, 0.06~0.14 was medium, >0.14 was large [28]. Reproducibility over time was estimated in the control group, calculated as the standard deviation of the differences between the two repeated measurements divided by the average of the averages of the repeated measurements and quoted as a percentage.

Results

Thirty OA patients (10 men and 20 women, aged 41-78, average age of 59.3 ± 10.1 years) participated in this study. One knee for each patient was scanned (Left =18, right =12). The KL scores of the knees based on previous x-ray data were score 0: n=1 (the contralateral knee had a score of 2); score 1, n= 4; score 2, n= 18; and score 3, n=7.

Table 1 summarizes the ROI data for the four regions evaluated in the central load bearing cartilage (cMF, MT, cLF, LT) evaluated at the three time points in the two groups of subjects. There was no significant

Table 1: Summary of ROI analysis (mean±SD).

	Full Thickness			Superficial Layer		
Active arm (n=19)	Baseline	3-Month	6-Month	Baseline	3-Month	6-Month
cMF	394±92	399±78	391±68*	373±77	394±74	378±55
MT	401±92	370±66	371±69*	375±72	377±68	364±76
cLF	444±96	448±79	451±82	406±103	377±67	374±78*
LT	418±70	402±66	392±76*	365±56	341±40	354±61
Control arm (n=10)						
cMF	455±75	456±77	466±100	430±102	446±77	446±90
MT	417±76	401±68	434±46	387±75	377±78	415±57
cLF	487±66	483±66	497±81	444±74	437±63	465±108
LT	429±86	458±108	461±89	375±77	411±101	416±69

* $p < 0.05$ compared to control group for the same ROI and the same time point

difference between the two groups at baseline and no significant difference in any group over time. There were few regions at 6 month time point that showed statistically significant differences between the two groups. However this is of minimal practical consequence. The effect size estimates showed that MT (0.164) had a large effect size and LT (0.096) had a medium effect size, but cMF (0.012) and CLF (0.012) had very small effect sizes. Correspondingly the required sample sizes for detecting change over time for MT and LT were 28 and 37, but jumped up to 379 and 323 for cMF and cLF respectively, for a 80% power at 0.05 significance level.

Table 2 shows that the reproducibility (coefficient of variation (CV %)) is very good for MT, cLF and LT with all <5%. The reproducibility of cMF between month 3 and month 0 is also good (CV<10%), but the variation increased to 14% and 19% for month 6 vs. month 0, and month 6 vs. month 3.

Figure 1 illustrates dGEMRIC images in the central medial condyle obtained in two representative subjects with KL score 2 at the three different time points. Note the significant difference in the relative T1 values, but more importantly the presence of full thickness cartilage defect in one while the other has cartilage thickness close to normal.

Table 3 summarizes the screening and baseline values of pain assessment by VAS pain scale as well as the patient global assessment. There were no statistically significant changes in either the G-F 20 or placebo group from baseline to either 3 months or 6 months and no differences between groups at any time point ($p > 0.05$ for all comparisons).

Discussion

Overall, there was little change in the mean dGEMRIC or pain indices over time in either the active or control group. There was no difference in the trends when only the superficial cartilage was evaluated (Table 1). However, the tibial cartilage exhibited a substantially larger effect size indicating that a sample size of 30-40 would have sufficient power to detect changes over time. The exact reason for the difference between tibial and femoral cartilage is not yet clear. It is also interesting that the cMF had lower reproducibility compared to all other compartments evaluated. Again, it is not yet clear what if any the significance of this observation, although this may be in part related to the loss of cartilage predominantly in this compartment in this group of patients.

Table 2: Reproducibility within the control group (coefficient of variation (CV%)).

	cMF	MT (log)	cLF (log)	LT (log)
Month 3 vs. month 0	9.10	1.54	1.91	2.75
Month 6 vs. month 0	19.08	2.85	3.04	2.10
Month 6 vs. month 3	14.26	2.43	3.25	2.57

Table 3: Summary of pain analysis (mean±SD).

VAS pain scale (mm)				
	Screening	**Baseline**	**3 months**	**6 months**
Active	44.8 ± 12.1	46.1 ± 22.4	47.4 ± 21.4	41.9 ± 29.5
Control	45.4 ± 16.1	49.7 ± 20.3	49.6 ± 24.8	42.6 ± 26.4

PGA (Likert)				
	Screening	**Baseline**	**3 months**	**6 months**
Active	4.3 ± 1.6	4.6 ± 1.8	4.7 ± 2.0	3.0 ± 2.4
Control	4.9 ± 1.7	5.1± 1.6	5.0 ± 1.6	4.4 ± 2.0

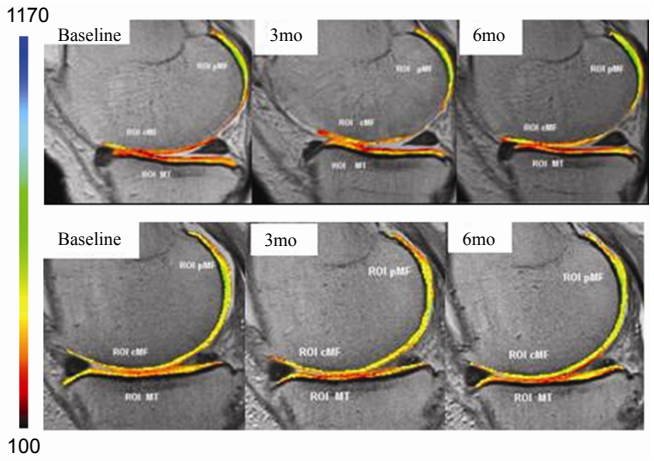

Figure 1: Many of the patients in this study had areas of denuded bone, as shown in the example above with a knee with KL score of 2 (Top). Another subject also with KL score of 2 but with no gross loss of cartilage is shown (Bottom). Both sets of images show little longitudinal change in dGEMRIC index over 6-mo in the active arm.

A recent pre-clinical study reported intervention with HA resulted in improved proteoglycan levels [29]. In a sheep model of surgically induced partial thickness articular cartilage lesion, administration of hyaluronic acid at days 0, 8, and 15 showed significant improvement in cartilage histological analysis and increased glycosoaminoglycan content compared to saline treated animals. A key factor in this preclinical study was that HA was administered early after knee injury.

We hypothesize that the lack of response in the current study may be related to the fact that the subjects included in the study had a relatively advanced stage of disease with respect to cartilage integrity even though efforts were specifically made to enroll patients with mild symptoms and radiographic changes. Thus, while KL=2 is generally considered to be mild OA clinically, cartilage imaging has shown extensive changes including full thickness loss of cartilage. Such a hypothesis is consistent with previous observations of disease modifying activity in humans with sodium hyaluronate where a response was observed only in the radiologically milder disease group [26]. Similarly in a recent meta-analysis, Wang et al. [30] found that patients older than 65 years with more advanced degenerative disease were less likely to respond to hyaluronan treatment than were their younger counterparts with less severe OA.

There are a number of other possible reasons for the lack of an observed primary effect in the current study: (i) The study was not adequately powered to detect a possible small (and unknown) effect size for the impact of hylan increasing proteoglycan content under these clinical conditions, (ii) hylan G-F 20 may not be effective as a disease modifying agent in patients with this level of disease, (iii) the time course may be too short to detect such an effect, and (iv) dGEMRIC, as implemented here, may not be sensitive to changes in the control group over 6 months, rendering it difficult to additionally detect a change from the active group if the effect is one of stabilizing the cartilage by hylan relative to degeneration in the control group.

In terms of clinical outcome metrics, since the subjects in this study were asked to continue the therapies they were currently taking at screening, with only 30 patients the study was not designed or powered to detect a potential incremental clinical benefit of adding hylan G-F 20.

Recommendations based on these results of this study include: (i) Utilizing the current results for power calculations for future studies. The stability of the control group has been determined from the current study; however, the level of possible change (improvement) with hylan G-F 20 is still unknown, and (ii) evaluating effects of administering hylan G-F 20 at earlier stages of OA. Alternatively, subjects with acute ligament or meniscal tears may be a better population to evaluate hylan G-F 20 and other potential disease modifying drugs. A recent pre-clinical study demonstrated changes in T2 of cartilage treated with hyaluronic acid following surgically induced ACL injury [31]. With the ability to detect and monitor early cartilage degeneration, the design of trials also need to change especially with respect to subject selection.

Competing Interests

The authors have no disclosures regarding financial and personal relationships with other people or organizations that could potentially and inappropriately influence (bias) their work and conclusions.

Contributions

PVP participated in the conception & design and interpretation of data. Assumed the lead role in drafting the article and was primarily responsible for securing the funding.

WL was primarily responsible for image acquisition, and analysis of data.

TS participated in the conception & design and were primarily responsible for subject recruitment, played a key role in the conception & design of the study, interpretation of data, and drafting the article.

NK was primarily responsible for collection, assembly and analysis of imaging data.

DB participated in the conception & design of the study, and was key in the interpretation of data and drafting the article.

All authors read and approved the final manuscript.

Acknowledgements and Funding

The authors thank Ms. Hongyan Du for the assistance with statistical analysis. Funding for this study included a grant from Genzyme Corporation.

References

1. Hootman JM, Helmick CG (2006) Projections of US prevalence of arthritis and associated activity limitations. Arthritis Rheum 54: 226-229.
2. Dougados M (2000) Sodium hyaluronate therapy in osteoarthritis: arguments for a potential beneficial structural effect. Semin Arthritis Rheum 30: 19-25.
3. Goldberg VM, Buckwalter JA (2005). Hyaluronans in the treatment of osteoarthritis of the knee: evidence for disease-modifying activity. Osteoarthritis Cartilage 13: 216-224.
4. Hunter DJ, Hellio Le Graverand-Gastineau MP (2008) How close are we to having structure-modifying drugs available? Rheum Dis Clin North Am 34: 789-802.
5. Ornetti P, Brandt K, Hellio-Le Graverand MP, Hochberg M, Hunter DJ, et al. (2009) OARSI-OMERACT definition of relevant radiological progression in hip/knee osteoarthritis. Osteoarthritis Cartilage 17: 856-863.
6. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC, Peterfy C (2006) Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. Osteoarthritis Cartilage 14: 46-75.
7. Hunter DJ, Le Graverand MP, Eckstein F (2009) Radiologic markers of osteoarthritis progression. Curr Opin Rheumatol 21: 110-7.
8. Burstein D, Gray M, Mosher T, Dardzinski B (2009) Measures of molecular composition and structure in osteoarthritis. Radiol Clin North Am 47: 675-86.
9. Gray ML, Burstein D, Kim YJ, Maroudas A (2008) 2007 Elizabeth Winston Lanier Award Winner. Magnetic resonance imaging of cartilage glycosaminoglycan: basic principles, imaging technique, and clinical applications. J Orthop Res 26: 281-291.
10. Bashir A, Gray ML, Burstein D (1996) Gd-DTPA2- as a measure of cartilage degradation. Magn Reson Med 36: 665-673.
11. Bashir A, Gray ML, Hartke J, Burstein D (1999) Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med 41: 857-865.
12. Juras V, Bittsansky M, Majdisova Z, Szomolanyi P, Sulzbacher I, et al. (2009) In vitro determination of biomechanical properties of human articular cartilage in osteoarthritis using multi-parametric MRI. J Magn Reson 197: 40-47.
13. Menezes NM, Gray ML, Hartke JR, Burstein D (2004) T2 and T1rho MRI in articular cartilage systems. Magn Reson Med 51: 503-509.
14. Nissi MJ, Toyras J, Laasanen MS, Rieppo J, Saarakkala S, et al. (2004) Proteoglycan and collagen sensitive MRI evaluation of normal and degenerated articular cartilage. J Orthop Res 22: 557-564.
15. Trattnig S, Mlynarik V, Breitenseher M, Huber M, Zembsch A, et al. (1999) MRI visualization of proteoglycan depletion in articular cartilage via intravenous administration of Gd-DTPA. Magn Reson Imaging 17: 577-583.
16. Zheng S, Xia Y, Bidthanapally A, Badar F, Ilsar I, et al. (2009) Damages to the extracellular matrix in articular cartilage due to cryopreservation by microscopic magnetic resonance imaging and biochemistry. Magn Reson Imaging 27: 648-655.
17. Laurent D, O'Byrne E, Wasvary J, Pellas TC (2006) In vivo MRI of cartilage pathogenesis in surgical models of osteoarthritis. Skeletal Radiol 35: 555-564.
18. Laurent D, Wasvary J, O'Byrne E, Rudin M (2003). In vivo qualitative assessments of articular cartilage in the rabbit knee with high-resolution MRI at 3 T. Magn Reson Med 50: 541-549.
19. Laurent D, Wasvary J, Rudin M, O'Byrne E, Pellas T (2003) In vivo assessment of macromolecular content in articular cartilage of the goat knee. Magn Reson Med 49: 1037-1046.
20. Wachsmuth L, Keiffer R, Juretschke HP, Raiss RX, Kimmig N, et al. (2003) In vivo contrast-enhanced micro MR-imaging of experimental osteoarthritis in the rabbit knee joint at 7.1T1. Osteoarthritis Cartilage 11: 891-902.
21. Watanabe A, Wada Y, Obata T, Ueda T, Tamura M, et al. (2006) Delayed gadolinium-enhanced MR to determine glycosaminoglycan concentration in reparative cartilage after autologous chondrocyte implantation: preliminary results. Radiology 239: 201-208.
22. Watterson JR, Esdaile JM (2000) Viscosupplementation: therapeutic mechanisms and clinical potential in osteoarthritis of the knee. J Am Acad Orthop Surg 8: 277-284.
23. Bagga H, Burkhardt D, Sambrook P, March L (2006) Longterm effects of intraarticular hyaluronan on synovial fluid in osteoarthritis of the knee. J Rheumatol 33: 946-950.
24. Ghosh P, Guidolin D (2002) Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent? Semin Arthritis Rheum 32: 10-37.
25. Waddell DD, Bert JM (2010). The use of hyaluronan after arthroscopic surgery of the knee. Arthroscopy 26:105-111.
26. Jubb RW, Piva S, Beinat L, Dacre J, Gishen P (2003) A one-year, randomised, placebo (saline) controlled clinical trial of 500-730 kDa sodium hyaluronate (Hyalgan) on the radiological change in osteoarthritis of the knee. Int J Clin Pract 57: 467-474.
27. Kimelman T, Vu A, Storey P, McKenzie C, Burstein D, et al. (2006) Three-dimensional T1 mapping for dGEMRIC at 3.0 T using the Look Locker method. Invest Radiol 41: 198-203.
28. Cohen J (1988) Statistical power analysis for the behavioral science. (2nd edition), Lawrence Erlbaum Associates, Hillsdale, NJ.
29. Kaplan LD, Lu Y, Snitzer J, Nemke B, Hao Z, et al. (2009) The effect of early hyaluronic acid delivery on the development of an acute articular cartilage lesion in a sheep model. Am J Sports Med 37: 2323-2327.
30. Wang CT, Lin J, Chang CJ, Lin YT, Hou SM (2004) Therapeutic effects of hyaluronic acid on osteoarthritis of the knee. A meta-analysis of randomized controlled trials. J Bone Joint Surg Am 86: 538-545.
31. Huang GS, Lee HS, Chou MC, Shih YY, Tsai PH, et al. (2010) Quantitative MR T2 measurement of articular cartilage to assess the treatment effect of intra-articular hyaluronic acid injection on experimental osteoarthritis induced by ACLX. Osteoarthritis Cartilage 18: 54-60.